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PATENT APPLICATION
Docket No.: 2984.1000-004

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PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant:	Yuan-Tsong Chen		
Application No.:	09/902,461	Group:	1654
Filed:	July 10, 2001	Examiner:	M. Meller
Confirmation No.:	6796		
For:	TREATMENT OF GLYCOGEN STORAGE DISEASE TYPE II		

CERTIFICATE OF MAILING
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BRIEF ON APPEAL

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Alexandria, VA 22313-1450

Sir:

This Brief on Appeal is submitted pursuant to the Notice of Appeal received in the U.S. Patent and Trademark Office on October 6, 2003, and in support of the appeal from the final rejections set forth in the Office Action mailed on June 3, 2003. The fee for filing a brief in support of an appeal is enclosed.

I. REAL PARTIES IN INTEREST

The real parties in interest are: Duke University, Erwin Road, Durham, North Carolina, Assignee of the entire right, title and interest in the subject application by virtue of an Assignment recorded on January 8, 2002 at Reel 012446, Frames 0057-0059; Synpac (North Carolina), Inc., 200 Meredith Drive, Durham, North Carolina, Licensee of the subject in the

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application; and Genzyme Corporation, One Kendall Square, Cambridge, Massachusetts, Sub-Licensee of the subject application.

II. RELATED APPEALS AND INTERFERENCES

Appellant, the undersigned Attorney, Assignee, Licensee and Sub Licensee are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-9 and 11-23 have been finally rejected, and a copy of these claims appears in Appendix A of this Brief. Claims 1, 8, 9, 21 and 22 were amended in the Amendment filed on September 16, 2002, and Claims 1, 11-15, 21 and 22 were amended in the Amendment filed on March 3, 2003. Claims 2-7 and 16-20 appear as originally filed. Claim 10 was canceled in the Amendment filed on September 16, 2002.

IV. STATUS OF AMENDMENTS

A Remarks After Final Rejection was submitted by facsimile on October 16, 2003, and was entered as indicated in the Advisory Action mailed from the U.S. PTO on November 18, 2003.

V. SUMMARY OF INVENTION

Appellant's invention includes a method of treating glycogen storage disease type II (GSD-II), also known as Pompe's Disease, in a human individual. In the method, a therapeutically effective amount of acid α-glucosidase (GAA) is administered to the individual periodically at an administration interval. The administration interval can be varied, depending on the severity of the symptoms and the needs of the individual. The GAA is human acid α-glucosidase (hGAA) produced in Chinese hamster ovary (CHO) cell culture. One advantage of production of GAA in CHO cell culture is that it is believed to yield hGAA having a glycosylation pattern which allows significant and efficient uptake of the hGAA in the desired tissues (heart and muscle) and resultant cleavage of glycogen, to treat GSD-II. See the

specification, for example, at p. 2, lines 22-25; p. 3, line 25, through p. 5, line 22; p. 5, line 23, through p. 6, line 13; p. 9, lines 4-23 and 24-28.

Appellant's invention includes a method of treating cardiomyopathy associated with GSD-II in a human individual by administering to the individual a therapeutically effective amount of hGAA periodically at an administration interval, wherein the hGAA was produced in CHO culture (Claim 21). Appellant's invention also includes a pharmaceutical composition comprising hGAA which was produced in CHO cell culture, in a container, the container having a label with instructions for administration of the composition for treatment of GSD-II. See the specification, for example, at the above pages as well as at p. 4, lines 23-26, and p. 10, lines 10-14.

VI. ISSUES

The following issues remain on appeal:

1) Whether Claims 1-9 and 11-23 are vague and indefinite under 35 U.S.C. §112, second paragraph, in view of the use of the word, "periodically;"

2) Whether Claims 1-4, 9, 21 and 23 are anticipated by Fuller *et al.* under 35 U.S.C. 102 (b);

3) Whether Claims 1-7, 11-18, 21 and 23 are anticipated by Fuller *et al.* under 35 U.S.C. 102 (b), or in the alternative, obvious over, Fuller *et al.* under 35 U.S.C. 103 (a);

4) Whether Claims 1-9 and 11-23 are obvious over Fuller *et al.* under 35 U.S.C. 103 (a); and

5) Whether Claims 1-9 and 11-23 are obvious over Bijvoet *et al.* in view of Fuller *et al.* under 35 U.S.C. 103 (a).

VII. GROUPING OF CLAIMS

With regard to issue 1, the claims do not stand or fall together: Claims 1-9, 11-21 and 23 are grouped together, and Claim 22 stands separately. With regard to the remaining issues (2 through 5), the claims do not stand or fall together. Based on the rejections set forth in the Office Action, made final, and for the purposes of this Appeal, Claims 1-9, 11-20 and 23 are grouped

together; Claim 21 stands separately; and Claim 22 stands separately. Discussion of the reasons why the claims of each of these group are separately patentable are included in the arguments presented below.

VIII. ARGUMENT

Issue 1

The Examiner rejected Claims 1-9 and 11-23 under 35 U.S.C. §112, second paragraph, stating "periodically" was vague and indefinite. This rejection is improper.

Words not specifically defined in the specification should be read as they would be by one of ordinary skill in the art. (M.P.E.P. Section 2111.01). Furthermore, a dictionary is an appropriate objective resource that can serve as a reliable source of information on established meaning that could be attributed to a claim term by one of skill in the art (see, e.g., Intellectual Property Development Inc. v. UA-Columbia Cablevision of Westchester Inc., 68 USPQ2d 1385 (CAFC 2003)). The Merriam-Webster Dictionary indicates that the term, "periodically," means "at regular intervals of time." A copy of the Dictionary entry was attached to the Remarks After Final Rejection (filed in the US PTO on October 16, 2003) as Exhibit A.

Further, the Specification provides several examples of what is intended by the word "periodically." See, e.g., p. 3, line 1, where it says "periodically (e.g., monthly, bimonthly, weekly, biweekly). See also p. 9, line 21 where it says "periodically (as distinguished from a one-time dose)". This language supports the ordinary definition of the word, periodically, as it is set forth in the dictionary. Thus, one of ordinary skill in the art would understand that "periodically" refers to administration at intervals of time. This is further supported by the additional claim language regarding administration "at an administration interval." Furthermore, one of ordinary skill in the art, given the Specification, would be able to determine the appropriate interval for a particular patient (see, e.g., p. 9, lines 24-29).

Appellant's Attorney also notes that the word, "periodically" has been used in the claims of granted U.S. patents relating to therapeutic application or administration of drugs or medications, without any further definition of the term in the specifications of such patents. See, for example, US Patents 4,735,802; 4,749,708; 4,761,417; 4,833,132; 5,292,754; 6,133,317; and 6,464,994. Copies of the claims of these Patents are attached in Appendix B.

For these reasons, the word "periodically" in Claims 1-9 and 11-23 does not make these claims vague and indefinite under 35 U.S.C. 112, second paragraph. Lastly, the word, "periodically," does not appear in Claim 22; therefore, this rejection is inapplicable to Claim 22.

Issue 2

The Examiner rejected Claims 1-4, 9, 21 and 23 under 35 U.S.C. §102(b) as being anticipated by Fuller *et al.*, Reference AV2. This rejection is also legally improper.

In order for a reference to anticipate a claim, each and every element at set forth in the claim must be found, either expressly or inherently described, in the reference (see, e.g., M.P.E.P. §2131). The following elements of the claims are neither expressly or inherently described by Fuller *et al.*: 1) administration of GAA *to a human individual*; 2) administration *periodically* and *at an administration interval*; 3) *treatment* of GSD-II in a human individual.

Fuller *et al.* describe preparation of recombinant GAA in CHO cell culture. Fuller *et al.* further indicate that the recombinant GAA was taken up in two types of cells from a patient having Pompe's disease: in cultured human skin fibroblasts after exposure to the enzyme for 12 hours, as well as in cultured human muscle cells after exposure to the enzyme for 24 hours. Lysosomal glycogen in the muscle cells was cleared, following addition of recombinant GAA to the culture medium of the cells. Fuller *et al.* do not describe administration to a human individual: they do not describe administration of GAA to anything other than cells in culture. Furthermore, Fuller *et al.* do not describe administration of GAA periodically, nor do they describe administration at an administration interval. In addition, Fuller *et al.* do not describe "treatment" of disease in an individual, as that term is described in the current Specification (see, e.g., p. 4, line 20 *et seq.*) and would be understood by one of ordinary skill in the art. Neither uptake of enzyme by cultured human fibroblasts *in vitro*, nor uptake of enzyme by cultured human skeletal muscle cells and subsequent processing of lysosomal glycogen in the muscle cells *in vitro*, both occurring in the short term (e.g., 12 to 24 hours), provides an indication whether administration of the GAA to a human patient, periodically, at an administration interval, will treat the disease. It is known in the art that *in vitro* cell culture conditions differ significantly from *in vivo* conditions: for example, when enzyme is administered *in vivo* by intravenous infusion, the muscle cells don't come into direct contact with enzyme as they do in cell culture.

Furthermore, the endothelial barrier, as well as the interstitial connective tissue, must be passed *in vivo*. See, for example, Reuser *et al.* (*Eur. J. Pediatr.* 161:S106-S111 (2002); a copy of which was attached to the Remarks After Final Rejection as Exhibit C) .

Claim 21 differs from Claims 1-4, 9 and 23 in that it pertains specifically to treatment of cardiomyopathy associated with GSD-II. Fuller *et al.* does not describe the impact of administration of enzyme to cardiac cells. Neither uptake of enzyme by cultured human fibroblasts, nor uptake of enzyme by cultured human skeletal muscle cells and subsequent processing of lysosomal glycogen in the muscle cells, as taught by Fuller *et al.*, provides any indication about the effects of the enzyme on cardiac cells. Furthermore, even if Fuller *et al.* did provide *in vitro* data regarding cardiac cells, such data would not indicate whether human heart cells *in vivo* would be able to uptake enzyme upon administration of the GAA to a patient and treat cardiomyopathy, because it is known in the art that *in vitro* cell culture conditions differ significantly from *in vivo* conditions, as stated above.

Despite the Examiner's statements, Fuller *et al.* do not teach each and every aspect of the claimed invention. Therefore, the claims are not anticipated by the teachings of Fuller *et al.*

<u>Issue 3</u>

The Examiner rejected Claims 1-7, 11-18, 21 and 23 under 35 U.S.C. §102(b) as anticipated by, or in the alternative, under 35 U.S.C. §103(a) as obvious over, Fuller *et al.*

The discussion regarding anticipation by Fuller *et al.* above similarly applies to the anticipation rejection of these claims. The rejection is incorrect.

With regard to the obviousness rejection, Fuller *et al.* state that they believe that the precursor GAA "will be a useful candidate for replacement therapy in GSD II patients."

In order for a reference to provide a legally sufficient basis for an obviousness rejection under Section 103, the reference must contain teachings to a person of ordinary skill that provide both (1) a motivation or suggestion to try and (2) a basis for a reasonable expectation of success. (see, e.g., M.P.E.P. §2143). Fuller, *et al.*, provide neither.

In regard to a suggestion to try, Fuller, *et al.* was published in 1995. Yet, despite the lack of any available treatment for this terrible disease, there are no published reports that *in vivo* research was attempted until Dr. Chen did so, about 5-6 years later. Those skilled in the art

looking for a treatment for Pompe's Disease clearly did not see the teachings of Fuller, *et al.* as a suggestion to treat humans having this disease with periodic administrations of a hGAA produced in CHO cells, as was done by Dr. Chen. Furthermore, as discussed above, Fuller *et al.* provide no motivation to administer enzyme periodically, at an administration interval, as they do not teach administration of enzyme more than once, nor do they suggest that periodic administration should be performed, or that administration at an interval should be used.

Assuming *arguendo* that one of ordinary skill in the art was motivated to try to treat Pompe's disease by this teaching of Fuller *et al.*, the current invention would nevertheless not have been obvious, because one of ordinary skill in the art would not have had a reasonable expectation that treatment would be successful.

The terrible effects of Pompe's disease, failure of other alternative treatments, and Dr. Chen's successful treatment are described by the Muscular Dystrophy Association (MDA) in the publication QUEST (Volume 10, Number 2, March/April 2003). A copy of this publication was left as a courtesy with the Examiner during the interview on September 17. A copy was also attached to the Remarks After Final Rejection as Exhibit B and is attached to this Brief as Appendix C.

Pompe's disease is a rare disease which is extremely devastating for the afflicted individuals; infants with the disease are not expected to live beyond one year of age. The disease has been known for about seventy years, since the early 1930's, and the enzyme deficiency has been known for about forty years, since the 1960's. And yet, many attempts at treatment of the disease by administration of replacement enzyme have failed. See, for example, Van der Ploeg *et al.* (*J. Clin. Invest* 87:513-518 (1991), cited in IDS as reference AW3, which lists several references that indicate that attempts at enzyme replacement therapy have failed); Williams *et al.* (Birth Defects: Original Article series Volume XVI, no. 1, pp. 415-423 (1980), cited in IDS as reference AW, which states that a preliminary trial to treat a terminally ill patient with Pompe disease was not clinically successful); and de Barsy *et al.* (Birth Defects: Original Article Series, Vol IX. No. 2, pp. 184-190(1973), cited in IDS as reference AU2, which states that no conspicuous morphologic or clinical improvements were noted after an attempt to treat a patient with enzyme; that no morphologic or biochemical evidence of replacement therapy had been

obtained to date; and that it appeared that the enzyme was not being transported to the relevant places in the body).

Dr. Chen demonstrated successful treatment of this genetic disease by administration of the enzyme to a human individual. As described in detail in the Example in the application, periodic administration of GAA produced in Chinese hamster ovary cells to three separate patients, resulted in significant amelioration of symptoms associated with the disease, as well as delay in onset of more severe symptoms. For example, significant improvements in cardiac parameters were noted in all patients; pulmonary function and skeletal muscle functions improved and remained normal in one patient; neurologic and developmental characteristics were either improved, or remained normal. The successful reversal of certain symptoms in all patients, as well as the normal muscle functions, neurologic and developmental characteristics of the third patient, were highly significant because it was previously unknown whether human symptoms could be alleviated or whether normal development could be achieved by administration of GAA. Without treatment, these children were expected to die; as reported in the attached MDA article, most infants with the disease aren't expected to live to one year of age. Dr. Chen's invention, on the other hand, is a successful treatment of an otherwise fatal genetic disease affecting heart and muscle tissues. The unexpected nature of this success is further emphasized in the Declaration under 37 C.F.R. §1.132 of Dr. Chen (the "Appellant"), previously submitted.

Furthermore, as noted above, the teachings of Fuller *et al.* provide no information on whether heart cells would be able to uptake and use the enzyme upon administration *in vivo*. With regard to the method of Claim 21, one of ordinary skill in the art would not have known or expected that administration of the enzyme to a human individual would result in successful treatment of cardiomyopathy associated with this genetic disease.

In view of the long-felt need for a treatment and the failure of others to treat Pompe's disease successfully, including attempts to treat the disease by enzyme replacement, one skilled in the art would not have had a reasonable expectation of success from the teachings of Fuller, *et al.* This, coupled with Dr. Chen's unexpected success, make the claimed invention non-obvious over the teachings of Fuller *et al.* under 35 U.S.C. 103 (a).

Issue 4

The Examiner also rejected Claims 1-9 and 11-23 under 35 U.S.C. §103(a) as being obvious over Fuller *et al.*, stating specifically that it would have been obvious to use an immunosuppressant with the enzyme to treat the disease, so that the body would be less likely to reject the enzyme and more readily use it to treat disease. The only claims that recite an immunosuppressant are Claims 19 and 20. Thus, it would seem that the rejection should only apply to these claims.

The M.P.E.P. indicates that "in considering the disclosure of a reference, it is proper to take into account not only specific teachings of the reference, but also the inferences which one skilled in the art would reasonably be expected to draw therefrom" (M.P.E.P. 2144.01, quoting *In re Preda*, 401 F.2d 825, 826, 159 USPQ 342, 344 (CCPA 1968)). While Fuller *et al.* describe preparation of recombinant GAA in CHO cell culture, and indicate that the recombinant GAA was taken up in two types of cells from a patient having Pompe's disease (cultured human skin fibroblasts and cultured human muscle cells), Fuller *et al.* do not teach or suggest that rejection of enzyme *in vivo* would be a concern; in fact, they do not describe use of the enzyme *in vivo* at all. Because there are no immune cells in the cell culture used by Fuller *et al.*, one of ordinary skill in the art would not expect or infer any results regarding an immune response to the enzyme. Thus, given the teachings of Fuller *et al.*, one of ordinary skill in the art would not be expected to draw an inference that rejection of enzyme *in vivo* would occur at all, as there is no teaching regarding any activity of the enzyme *in vivo* and no teaching regarding any immunoreactivity of the enzyme. In view of these considerations, the invention of Claims 19 and 20 would not have been obvious over the teachings of Fuller *et al.*

Issue 5

The Examiner rejected Claims 1-9 and 11-23 under 35 U.S.C. 103(a) as being unpatentable over Bijvoet *et al.*, Reference AR2, in view of Fuller *et al.*, stating that it would have been obvious to use the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*

Bijvoet *et al.* describe production of transgenic recombinant hGAA in mouse milk; administration of a single dose of GAA to GSD-II knockout mice, and a resultant increase of enzyme activity in homogenized heart and skeletal muscle samples after two days; and the uptake

of the enzyme by cultured human fibroblasts. Bijvoet *et al.* do not describe administration of GAA to a human individual, as is required by the claims; in addition, Bijvoet *et al.* do not teach or suggest administration of GAA periodically, at an administration interval. Furthermore, they do not describe "treatment" of disease as it is described in the Specification and would be understood by one of ordinary skill in the art.

One of ordinary skill in the art, given the teachings of Bijvoet *et al.*, would not have been motivated to look to the teachings of Fuller *et al.* regarding enzyme produced in CHO cell culture; in fact, Bijvoet *et al.* teach away from use of enzyme produced in CHO cells: they indicate that high production costs associated with use of enzyme produced in CHO cells are a significant concern and discuss experiments designed to provide proof of principle for obtaining enzyme by other means (Bijvoet *et al.*, p. 1820, "Discussion").

Even assuming *arguendo* that the teachings of Bijvoet *et al.* were combined with the teachings of Fuller *et al.*, one of ordinary skill in the art would not have obtained the present invention. One of ordinary skill in the art, using the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*, would have been motivated only to administer a single dose of the enzyme, and not to administer enzyme periodically at an administration interval.

Furthermore, given the teachings of Bijvoet *et al.* in combination with the teachings of Fuller *et al.*, one would not have had a reasonable expectation of successfully treating a human individual. As discussed above, there was a long-felt need for a treatment and significant failures by others to achieve treatment of Pompe's Disease by any means, including administration of enzyme. One of ordinary skill in the art would not have known, given the teachings of Bijvoet *et al.* regarding uptake of enzyme by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme, whether periodic administration, at an administration interval, to a human with Fuller *et al.*'s GAA, would successfully "treat" the patient, as treatment is described in the Specification and would be understood by one of ordinary skill in the art.

Furthermore, Even assuming *arguendo* that the teachings of Bijvoet *et al.* were combined with the teachings of Fuller *et al.*, one of ordinary skill in the art would not have obtained the present invention as set forth in Claim 21. One of ordinary skill in the art, using the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*,would not have known whether administration *in*

vivo would successfully treat cardiomyopathy, because one would not have had a reasonable expectation of success. The teachings of Bijvoet *et al.* describe uptake of enzyme by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme; if these teachings were combined with the teachings of Fuller *et al.* regarding the enzyme, the combination does not provide a reasonable expectation that administration of the enzyme periodically to a patient at an administration interval will, in fact, treat cardiomyopathy. Bijvoet *et al.* describe increased activity in homogenized mouse heart and muscle tissue; these experiments do not indicate whether the enzyme has located to the relevant cells (e.g., myocytes), that will allow it to treat disease (e.g., by decreasing glycogen and/or decreasing symptoms). In fact, as supported by Dr. Chen's Declaration, one of ordinary skill in the art would expect that intravenous administration of enzyme would result in the presence of the enzyme in the blood stream and endothelium of blood vessels, rather than in the desired target cells. Furthermore, even if the enzyme located to the desired cells, Bijvoet *et al.* do not demonstrate a decrease of glycogen or other correction of symptoms.

As discussed above, there was a long-felt need for a treatment of Pompe's Disease (GSD-II) and significant failures by others in the art to achieve treatment. One of ordinary skill in the art would not have known, given the teachings of Bijvoet *et al.* regarding uptake of enzyme (produced in milk) by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme, whether periodic administration, at an administration interval, to a human individual, of the GAA as taught by Fuller *et al.,* would in fact result in successful treatment of cardiomyopathy as set forth in Claim 21.

With regard to Claim 22, Fuller *et al.* in combination with Bijvoet *et al.* do not teach or suggest a pharmaceutical composition comprising human acid α- glucosidase, wherein the human acid α- glucosidase was produced in chinese hamster ovary cell culture, in a container, the container having a label containing instructions for administration of the composition for treatment of GSD-II. As discussed in detail above, the combination of Fuller *et al.*, and Bijvoet *et al.* does not describe treatment of GSD- II, as the term "treatment" is understood by one of ordinary skill in the art. Therefore, it would not have been obvious to one of ordinary skill in the art to prepare a pharmaceutical composition in a container having a label with instructions for administration of the composition for treatment of the disease, as one would not have known

whether it was even possible to treat the disease: one would not have been able to prepare the label.

In view of these considerations, the claimed invention would not have been legally obvious under Section 103 over the teachings of Bijvoet *et al.* in combination with Fuller *et al.*

CONCLUSION

For the reasons presented above, Appellant's Attorney respectfully requests the Board of Patent Appeals and Interferences to reverse all of the rejections in the Office Action of June 3, 2003.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

David E. Brook, RN 22592

By for Elizabeth W. Mata

Elizabeth W. Mata
Registration No. 38,236
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

Date: 1/6/03

APPENDIX

1. (Twice Amended) A method of treating glycogen storage disease type II in a human individual having glycogen storage disease type II, comprising administering to the individual a therapeutically effective amount of human acid glucosidase periodically at an administration interval, wherein the human acid α- glucosidase was produced in chinese hamster ovary cell cultures.

2. The method of Claim 1, wherein the glycogen storage disease type II is infantile glycogen storage disease type II.

3. The method of Claim 1, wherein the glycogen storage disease type II is juvenile glycogen storage disease type II.

4. The method of Claim 1, wherein the glycogen storage disease type II is adult-onset glycogen storage disease type II.

5. The method of Claim 1, wherein the therapeutically effective amount of human acid α-glucosidase is less than about 15 mg of acid α-glucosidase per kilogram of body weight of the individual.

6. The method of Claim 5, wherein the therapeutically effective amount of human acid α-glucosidase is about 1-10 mg of acid α-glucosidase per kilogram of body weight of the individual.

7. The method of Claim 5, wherein the therapeutically effective amount of human acid α-glucosidase is about 5 mg of acid α-glucosidase per kilogram of body weight of the individual.

8. (Amended) The method of Claim 1, wherein the human acid glucosidase is recombinant human acid glucosidase that has been produced in chinese hamster ovary cell cultures.

9. (Amended) The method of Claim 1, wherein the human acid glucosidase is a precursor of recombinant human acid glucosidase that has been produced in chinese hamster ovary cell cultures.

11. (Amended) The method of Claim 1, wherein the administration interval is monthly.

12. (Amended) The method of Claim 1, wherein the administration interval is bimonthly.

13. (Amended) The method of Claim 1, wherein the administration interval is weekly.

14. (Amended) The method of Claim 1, wherein the administration interval is twice weekly.

15. (Amended) The method of Claim 1, wherein the administration interval is daily.

16. The method of Claim 1, wherein the human acid α-glucosidase is administered intravenously.

17. The method of Claim 1, wherein the human acid α-glucosidase is administered intramuscularly.

18. The method of Claim 1, wherein the human acid α-glucosidase is administered intrathecally or intraventricularly.

19. The method of Claim 1, wherein the human acid α-glucosidase is administered in conjunction with an immunosuppressant.

20. The method of Claim 19, wherein the immunosuppressant is administered prior to any administration of human acid α-glucosidase to the individual.

21. (Twice Amended) A method of treating cardiomyopathy associated with glycogen storage disease type II in a human individual having glycogen storage disease type II, comprising administering to the individual a therapeutically effective amount of human acid glucosidase periodically at an administration interval, wherein the human acid glucosidase was produced in chinese hamster ovary cell culture.

22. (Twice Amended) A pharmaceutical composition comprising human acid glucosidase, wherein the human acid glucosidase was produced in chinese hamster ovary cell culture, in a container, the container having a label containing instructions for administration of the composition for treatment of glycogen storage disease type II.

23. The method of Claim 1, wherein the administration interval is varied over time.

EXHIBIT A

Main Entry: pe·ri·od·i·cal·ly
Pronunciation: "pir-E-'ä-di-k(&-)lE
Function: adverb
Date: 1646
1 : at regular intervals of time
2 : from time to time : FREQUENTLY

1. Pronunciation Key

EXHIBIT B

is kept in place undisturbed until completely dry. Optionally, it can be removed aseptically by suitable means such as washing and drying the skin. Conveniently the mask can be applied and set before bedtime in the evening and during the night, allowed to fall off or slough off as when completely dry. The second application of the mask typically can be undertaken in a short period, e.g. with the next few hours. To a considerable extent, the effectiveness of the therapy depends on the number of successive applications. Typically, a beneficial result can be observed within several days, usually within about 3 to 5 days, in a regimen where the treatment is given each day or preferably each night, as described. The papules first lose their edematous, erythematous appearance, they then shrink and finally disappear. For difficult, deepseated inflammatory lesions, the progressive healing starts as the reduction of firmness, then shrinkage of lesions, and the eventual disappearance of the lesions, with absence of or minimal scarring.

In another aspect, the invention concerns a dermatological composition adapted for topical treatment of dermatoses of the kind described, comprising a sterile homogeneous therapeutic paste made by mixing a water component and dried calcium sulfate in fine powder form that essentially is an odorless, tasteless hemihydrate. In one preferred embodiment, the composition is packaged with the water component and the dried powder component in separate sealed containers. The paste is of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing. The water component in one preferred embodiment optionally contains a water soluble thickener such as sodium carboxymethyl cellulose in an amount sufficient to cause the water component to gel and prevent the water component from flowing. The water component suitably may contain a self sterilizing agent which may be conventional and preferably is trichlorcarban, oxychlorosene sodium or triclosan constituted in dilute solution e.g. 0.1–0.2%. Suitable topical anti-infectives are described in The Nurses Guide to Drug Therapy, Chapter 88, Prentice-Hall, Inc., 1984.

The paste in one preferred embodiment includes a per se effective amount of a compatible anodyne such as zinc oxide. As constituted if necessary with buffering, the paste is essentially at neutral pH.

A preferred compositon for treating dermatoses is a smooth workable paste made by mixing sterile water with heat-sterilized calcium sulfate hemihydrate, in the weight ratio of 4 parts to about one part; optionally with thickener, buffer, anti-infective agent and/or anodyne.

Having thus described my invention, the embodiments in which an exclusive property or privilege is claimed are defined as follows:

1. A method of treating dermatoses that are characterized by lesion sites, exudate, and chronic inflammation of the sebaceous glands and follicles of the skin, including the steps of:

(a) applying a sterile homogeneous settable therapeutic paste composition consisting essentially of a mixture of water and dried calcium sulfate, to the skin area comprising the lesion sites in a co-extensive overlying soft mask or layer,

(b) allowing the mask to set until hard and to remain in place on the skin for a time sufficient to become dry and achieve absorption and adsorption of exudate,

(c) allowing the mask to slough off or optionally removing the thus sorbed exudate by aseptic removal of the mask from the skin;

and, if necessary repeating the regimen of steps a, b and c periodically until remission of the lesions is achieved.

2. The method of treating acne according to claim 1 where the regimen of steps a, b and c is carried out once daily for several days until remission of the lesions is achieved.

3. The method of treating acne according to claim 1 where the regimen of steps a, b and c is carried out once each night for several nights until remission of the lesions is achieved.

4. A dermatological composition adapted for topical treatment of dermatoses that are characterized by lesion sites, exudate and chronic inflammation of the sebaceous glands and follicles of the skin, consisting essentially of a sterile homogeneous therapeutic paste made by mixing a water component containing a self-sterilizing agent in an amount sufficient to maintain sterility of the composition, and dried calcium sulfate in fine powder form that essentially is an odorless tasteless hemihydrate, the paste being of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing.

5. A composition according to claim 4 where the self-sterilizing agent is selected from trichlorcarban, oxychlorosene sodium and triclosan.

6. A composition according to claim 4 where the paste includes a per se effective amount of a compatible anodyne agent.

7. A composition according to claim 4 where the paste is essentially at neutral pH.

8. A dermatological composition adapted for topical treatment of dermatoses that are characterized by lesion sites, exudate and chronic inflammation of the sebaceous glands and follicles of the skin, consisting essentially of a sterile homogeneous therapeutic paste made by mixing a water component and dried calcium sulfate in fine powder form that essentially is an odorless tasteless hemihydrate, the paste being of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing, the water component containing water soluble carboxymethyl cellulose in an amount sufficient to cause the water component to gel and prevent the water component from flowing.

9. A composition according to claim 8 where the paste includes a self-sterilizing agent.

* * * * *

These dogs were instrumented with an electromagnetic flowmeter placed around the ascending aorta to measure cardiac output, with catheters in the left ventricle and the aorta for recording of their respective pressure. All these parameters together with left ventricular dP/dt and lead aVF of the electrocardiogram were recorded during the experiments continuously at a speed of 1 mm/second and at 15 minute intervals at a speed of 200 mm/second.

Coreximine was given orally at doses of 0.25, 0.5 and 5 mg/kg. Each dose was studied in 6 dogs. At a dose of 5 mg/kg the total amount of the compound delivered approximates the amount delivered by infusion at 0.2 mg/kg/min for 30 minutes (6 mg/kg).

The dose of 0.25 mg/kg was only mildly effective. At the dose of 0.5 mg/kg, the left ventricular dP/dt increased by about 30% from 2618 ± 221 to 3556 ± 371 mm Hg/sec ($P<0.05$). Cardiac output increased significantly from 2.4 ± 0.1 to 2.9 ± 0.1 l/min ($P<0.01$) and left ventricular and diastolic pressure fell from 6.7±0.8 to 5.8±0.6. At the same time mean arterial pressure increased by 20 mm of Hg while heart rate increased from 73 to 94 beats per minute. Therefore, oral coreximine, even at a very low dose, is a positive inotropic agent improving left ventricular performance and is well absorbed orally. It should be noted, however, that there was some increase in heart rate and blood pressure.

At the higher dose of 5 mg/kg, which corresponds to 0.17 mg/kg/min, the dP/dt increased from 2559±76 to 4630±378 mm Hg/sec ($P<0.005$), an increase of about 80%. The systolic arterial pressure increased from 125 to 168 mm Hg while the diastolic arterial pressure increased from 78 to 106 mm Hg. Heart rate increased from 83 to 133. Therefore, while heart rate and blood pressure increased, the dP/dt increased markedly. The cardiac output increased from 2.2±0.1 to 2.6±0.1 ($P<0.005$) and total peripheral resistance increased by 22%. Left ventricular and-diastolic pressure fell from 6.4±0.6 to 4.4±0.7 ($P<0.05$).

Thus, coreximine increased strikingly contractility, decreased the preload and increased cardiac output thus augmenting performance while increasing arterial pressure and heart rate.

The data, which shows an increase in heart rate and increase in arterial pressure when coreximine is administered orally in contrast to a decrease in these two effects when the drug is administered intravenously suggests that the increase in the heart rate and arterial pressure is due to the bitterness of the berberine derivatives or some other outside influence. In the above experiments the natural bitterness of coreximine was not masked by conventional means known to those skilled in the art.

Next, coreximine was given orally to dogs that were pretreated with propanolol at a dose of 1 mg/kg. Propanolol was given to verify whether coreximine has a beta adrenergic agonist activity, specifically to verify whether the increased in contractility measured by dP/dt max and dP/dt at 40 mm Hg of developed pressure would disappear. It was found that the increase in contractility does not disappear after this beta blockade and therefore the increase in contractility is not due to beta angonist effects either of the drug itself or other external influences. The increases in blood pressure also were not nullified; however, increases in blood pressure are alpha and not beta mediated.

In another experiment, 13-allylberberine bromide was given orally to conscious dogs at a dose of 5 and of 50 mg/kg. Each dose was given to 5 dogs. The compound is effective orally when given 50 mg/kg but not when given 5 mg/kg. When given 50 mg/kg it increased dP/dt significantly from 2561 to 3391 mm Hg/sec, showing a marked positive inotropic property. Also, cardiac output increased from 2.3 to 2.7 l/min and left ventricular end-diastolic pressure fell from 5.S to 4.5 mm Hg showing an improvement in ventricular performance and a reduction in preload. Heart rate increased from 69 to 81 and mean arterial pressure from 90 to 116 mm Hg. The increase in heart rate and arterial pressure again suggests the influence of the bitterness of the drug.

Moreover, when 2 doses of 13-allylberberine bromide 50 mg/kg were given orally with an interval of 30 minutes between them dP/dt increased from 2500 to 4600 mm Hg, an increase of 84% showing that higher or multiple dose may be more effective.

Similarly other pharmaceutically acceptable salts of 13-allylberberine produce equivalent effects.

13-Methylberberine citrate given 50 mg/kg orally in 2 dogs increased left ventricular dP/dt by an average of 27% suggesting that this compound was absorbed when given orally. When it was given intravenously 0.2 mg/kg/min for 30 minutes its effect was similar suggesting around a 10:1 intravenous to oral ratio of effectiveness.

Berberine tartrate (5 mg/kg) given orally to 2 dogs increased left ventricular dP/dt by 17%.

None of the above mentioned compounds caused arrhythmias. The compounds of the instant invention have either an antiarrhythmic effect or no obserable effect on arrhythmias at the dosage at which the compounds are administered in the above experiments.

I claim:

1. A therapeutic method of increasing the contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of coreximine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

2. The therapeutic method of claim 1 wherein the compound is administered periodically.

3. The therapeutic method of claim 1 wherein the composition is administered by intravenous infusion.

4. A therapeutic method of increasing the contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering orally to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of coreximine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

5. The therapeutic method of claim 4 wherein the compound is administered periodically.

6. The method of claims 1, 2, 3, 4 or 5 wherein the compound is administered in a dosage in the range of about 0.2 to 5 mg/kg.

* * * * *

TABLE XIV-continued

Effect of Berberine on Ejection Fraction

		Ejection fraction (quadruplicate reading)				Mean	Average
5 min	30 min	.43	.47	.45	.42	.44	
infusion	45 min	.35	.39	.37	.39	.38	
Berberine	5 min	.45	.46	.50	.43	.46	0.40 ± 2
0.7 mg/kg/min	15 min	.37	.39	.37	.35	.37	($p < 0.05$)
	30 min	.41	.41	.38	.38	.40	
	45 min	.36	.39	.42	.35	.38	

TABLE XV

Effect of Berberine (0.2 mg/kg/min) on Ejection Fraction (as done by MUGR) in Conscious Dogs with Acute Left Ventricular Failure (LVF).

	Before LVF	LVF	During Berberine Infusion	30 min after Berberine Infusion
Dog #				
1	45	24	38	33
2	56	34	51	41
3	53	35	49	44
4	58	20	31	30
5	55	21	35	30
6	45	28	39	33
Average ± SE	55 ± 2	27 ± 2	40 ± 3	35 ± 2
	$p < 0.001$		$p < 0.001$	
			$p < 0.001$	

TABLE XVI

Effects of Berberine and Ouabain on Hemodynamics in Dogs with Acute Heart Failure

	Control		Oubain		Berberine	
	Before	ΔChange	Before	ΔChange	Before	ΔChange
Heart rate (beats/min)	136 ± 4	0 ± 3	124 ± 5	−3 ± 1*	126 ± 12	−8 ± 2*
Systolic AP (mmHg)	124 ± 9	−1 ± 4	115 ± 6	1 ± 3	118 ± 7	−4 ± 3
Diastolic AP (mmHg)	97 ± 9	−1 ± 4	84 ± 7	3 ± 2	90 ± 7	−9 ± 3*
Mean AP (mmHg)	111 ± 9	−3 ± 4	98 ± 7	0 ± 2	102 ± 7	−6 ± 2*
Pulse pressure (mmHg)	26 ± 1	0 ± 1	31 ± 3	−1 ± 2	28 ± 2	5 ± 3
LV dP/dt (mmHg/sec)	1628 ± 132	−72 ± 64	1700 ± 80	178 ± 61*	1572 ± 80	440 ± 91 ‖ **
LV EDP (mmHg)	14.1 ± 0.5	0.4 ± 0.5	13.0 ± 1.8	−2.0 ± 1.1	13.3 ± 0.8	−5.0 ± 0.8 ‖ **
Cardiac output (liter/min)	1.77 ± 0.28	−0.22 ± 0.08*	1.84 ± 0.16	−0.07 ± 0.18	1.76 ± 0.15	0.17 ± 0.06 **
Stroke volume (ml/beat)	12.9 ± 1.8	−1.4 ± 0.5	14.8 ± 1.1	−0.2 ± 1.4	14.6 ± 1.9	2.7 ± 1.1 *
TPR ($dyn.sec.cm^{-5}$)	5943 ± 1124	601 ± 466	4495 ± 551	10 ± 491	4915 ± 559	−852 ± 156 **

AP = Aortic pressure; LV dP/dt = Maximal rate of rise of left ventricular pressure; LV EDP = left ventricular end diastolic pressure; TPR = Total peripheral vascular resistance; * = $p < 0.05$ and ** = $p < 0.005$ for paired t-test between values before and after intervention; = $p < 0.05$ and = $p < 0.01$ for unpaired t-test between control group and treated groups; ‖ = $p < 0.05$ for unpaired t-test between berberine HCl and oubain treated groups.

TABLE XVII

Effect of Berberine, Oubain and the Combination of Both Peak LV dP/dt (numbers are percent increase compared to before treatment).

	Time after Treatment					
Drug Intervention	5 min	10 min	15 min	20 min	25 min	30 min
Berberine alone	30.2 ± 4.1	36.2 ± 3.5	39.2 ± 3.3	42.6 ± 4.9	38.8 ± 5.4	40.5 ± 6.7
Ouabain alone	17.1 ± 2.1	24.0 ± 2.8	33.8 ± 3.8	36.1 ± 4.3	44.0 ± 6.3	49.0 ± 7.3
Ouabain and Berberine	xxx	xxx	xxx	xxxx	xxxx	xxxx
	87.1 ± 10.0	82.0 ± 12.8	87.5 ± 13.3	95.4 ± 16.0	98.2 ± 16.5	97.6 ± 14.7
	ooo	ooo	ooo	oooo	oooo	oooo

Ouabain and Berberine vs. Berberine alone = $p < 0.005$ ooo
$p < 0.000$ oooo
Ouabain and Berberine vs. Ouabain alone = $p < 0.005$ xxx
$P < 0.001$ xxxx

I claim:

1. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of berberrubine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing administration.

2. The therapeutic method of claim 1 wherein the compound is administered periodically.

3. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of tetrahydropalmatine and its pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing administration.

4. The therapeutic method of claim 3 wherein the compound is administered periodically.

5. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof a composition which comprises a pharmaceutically acceptable carrier and a cardiac glycoside and in conjunction therewith, in an amount effective to cause a positive inotropic effect, a composition which comprises a pharmaceutically acceptable carrier and a compound selected from the group consisting of berberrubine, tetrahydropalmatine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

6. The therapeutic method of claim 5 wherein the compound is administered periodically.

7. The therapeutic method of claim 5 wherein the compound is berberrubine or its pharmaceutically acceptable salts.

8. The therapeutic method of claim 5 wherein the compound is tetrahydropalmatine or its pharmaceutically acceptable salts.

9. The therapeutic method of claim 5, 6, 7 or 8 wherein the cardiac glycoside is selected from the group consisting of ouabain, digoxin, digitoxin and delanoside.

10. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof a composition which comprises a pharmaceutically acceptable carrier and a cardiac glycoside and in conjunction therewith, in an amount effective to cause a positive inotropic effect, a composition which comprises a pharmaceutically acceptable carrier and a compound selected from the group consisting of berberine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing the administration of the composition comprising the cardiac glycoside and the compositiion comprising the compound.

11. The therapeutic method of claim 10 wherein the compound is administered periodically.

12. The therapeutic method of claim 10 or 11 wherein the cardiac glycoside is selected from the group consisting of ouabain, digoxin, digitoxin and delanoside.

13. The method of claims 1, 2, 3 or 4 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

14. The method of claims 1, 2, 3 or 4 wherein the compound is administered in a dosage in the range of about 0.02 to 0.7 mg/kg.

15. The method of claim 5 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

16. The method of claim 5 wherein the compound is administered in a dosage in the range of about 0.02 to about 0.7 mg/kg.

17. The method of claim 10 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

18. The method of claim 10 wherein the compound is administered in a dosage in the range of about 0.02 to 0.7 mg/kg.

* * * * *

treated eye) of 3 points at 2, 4 and 6 hours after application.

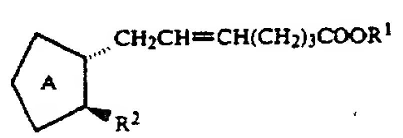
TABLE 11

	difference (mmHg)						
days	0	14	28	42	56	70	84
Sodium salt of 6	2.20	2.02	2.24	2.03	2.01	2.09	2.14
timolol maleate	2.73	2.54	1.10	1.26	1.20	1.26	1.22

It is known that the intraocular pressure-reducing effect of timolol maleate, which is most broadly used in clinical field, is lowered in association with the lapse of the application term. The results in Table 11 shows that the intraocular pressure-reducing effect of the sodium salt of the compound 6 is not lowered even if it is used in a long duration, which is an advantage of the compound of the present invention over timolol maleate.

What is claimed is:

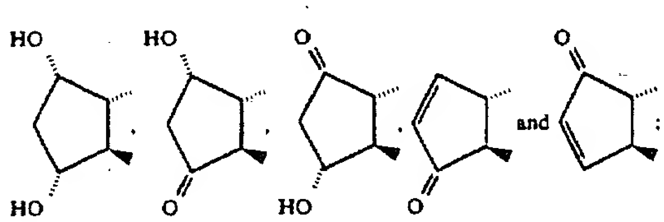
1. A method for treating hypertension or glaucoma in the eye comprising contacting the surface of the eye with a therapeutic amount of a 15-deoxyprostaglandin derivative of the formula (I):



(I)

in which

is a 5 membered ring which is selected from a group consisting of

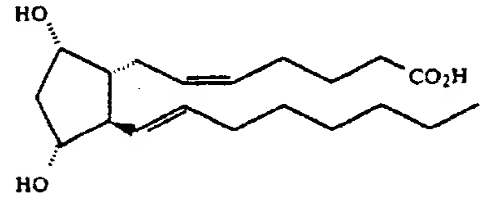


R^1 is hydrogen or lower alkyl;

R^2 is C_6–C_{12} alkyl, C_6–C_{12} alkenyl or C_6–C_{12} alkadienyl or a pharmaceutically acceptable salt thereof.

2. The method as claimed in claim 1, wherein the compound as indicated in claim 1 is periodically contacted with the surface of the eye.

3. The method as claimed in claim 1, wherein (5Z,13E,9S,11R)-9,11-dihydroxy-5,13-prostadiehoic acid of the formula (I):



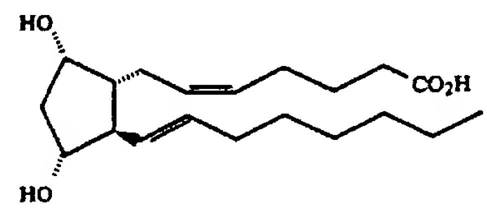
or a pharmaceutically acceptable salt or a lower salt alkyl ester is periodically contacted with the surface of the eye at a dose of 1 μg/eye/day to 1000 μg/eye/day.

4. A kit for delivery of a topical solution for treatment of hypertension or glaucoma in the eye which comprises:

(a) container having a solution including a compound of the formula (I), and

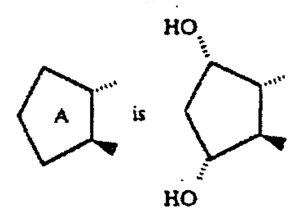
(b) means for topical delivery of said solution to the eye in a controlled dosage.

5. The kit as claimed in claim 4, wherein the compound is (5Z,13E,9S,11R)-9,11-dihydroxy-5,13-prostadienoic acid of the formula (I):



or a pharmaceutically acceptable salt or a lower alkyl ester.

6. The method as claimed in claim 1, wherein



7. The method as claimed in claim 1, wherein R^2 is C_6–C_{12} alkenyl.

8. The method as claimed in claim 7, wherein R^2 is C_8–C_{10} alkenyl.

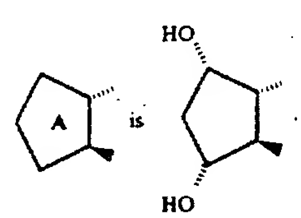
9. The method as claimed in claim 8, wherein R^2 is a straight-chain C_8–C_{10} alkenyl.

10. The method as claimed in claim 6, wherein R^2 is C_6–C_{12} alkenyl.

11. The method as claimed in claim 10, wherein R^2 is C_8–C_{10} alkenyl.

12. The method as claimed in claim 11, wherein R^2 is a straight-chain C_8–C_{10} alkenyl.

13. The kit as claimed in claim 4, wherein



14. The kit as claimed in claim 4, wherein R^2 is C_6–C_{12} alkenyl.

15. The kit as claimed in claim 14, wherein R^2 is C_8–C_{10} alkenyl.

16. The kit as claimed in claim 15, wherein R^2 is a straight-chain C_8–C_{10} alkenyl.

17. The kit as claimed in claim 13, wherein R^2 is a C_6–C_{12} alkenyl.

18. The kit as claimed in claim 17, wherein R^2 is C_8–C_{10} alkenyl.

19. The kit as claimed in claim 18, wherein R^2 is a straight-chain C_8–C_{10} alkenyl.

* * * * *

TABLE XI

VITAMIN B-6 (PYRIDOXINE) CONTENT OF SELECTED BAKED GOODS

Breads	Mg/100 G
Bagels: Plain, Onion, Poppy Seed, Sesame	.051
Bagels, Date Bran	.000
Bagels, Egg	.084
Bagels, Cinnamon Raisin	.000
Biscuits, Commercially Baked Plain or Buttermilk	.047
Cornbread	.113
Cracked Wheat Bread	.304
French and Vienna Bread	.043
Italian Bread	.048
Mixed Grains 7 Bread	.333
Oat Bran Bread	.000
Pumpernickel	.126
Rye Bread	.075
Wheat Bran Bread	.064
Whole Wheat Commercial Bread	.179

Source: U.S. Deptartment of Agriculture
Agriculture Handbook 8–18 Rev. 1992

TABLE XII

Dry Dog Food
Test Run Data

E325 Processing Conditions:	Run #1	Comment
Product	Dry Dog Food	
Feeder Speed (RPM)	11 rpm (420 lbs/hr)	
Feeder Speed (Hertz)	34.7	
Cond. Cyl. Temp (° F.)	206	
Cond. Cyl. Water (% gauge)	10	
Extruder Speed (RPM)	421	
Extruder Current (Amps)	24	
Extruder Water (% gauge)	0	
Extruder Steam Injection	0	
#2 Head Temp. (CW or ST)	CW	
#3 Head Temp. (CW or ST)	CW	
#4 Head Temp. (CW or ST)	CW	
#5 Head Temp. (° F.)	CW 79 deg F.	
#6 Head Temp. (° F.)	CW 129 deg F.	
Die Pressure (PSI)	350	
Knife Speed (Hertz)	55.9	
Dryer Temperature (° F.)	223	
Dryer Retention (minutes)	17.9	
Formula:	See Dry Feed formula 2.2% Oxalic acid solution added at 0.7 lbs/min	2.7# OA to 100# water
Comments:	The oxalic acid solution was made up in hot water (160° F.) and pumped into the conditioning cylinder	
Screw Configuration:		
#1 Screw	Single Flight Tapered Inlet	Straight Rib Head
#1 Steamlock	Spacer	
#2 Screw	Single Flight Uncut	Spiral Rib Head
#2 Steamlock	Spacer	
#3 Screw	Single Flight Uncut	Spiral Rib Head
#3 Steamlock	Spacer	
#4 Screw	Single to Double Flight Uncut	Straight Rib Head
#4 Steamlock	Small	
#5 Screw	2 Flight cut flight	Straight Rib Head
#5 Steamlock	Large	
#6 Screw	2 Flight cut flight cone	Spiral Rib Cone Head
Die Configuration:		
Spacer	1" thick	
Backup Die	No	
Dieplate	1 1/4" central insert die	#825440-3
Insert	1/4" round hole	#101-509

Thus, it will be appreciated that as a result of the present invention, a highly effective oxalic acid or oxalate composition and method is provided by which the principal object, among others, is completely fulfilled. It is contemplated, and will be apparent to those skilled in the art from the preceding description that modifications and/or changes may be made in the prescribed embodiments without departure from the present invention. Accordingly, it is expressly intended that the foregoing description is illustrative of preferred embodiments only, and not limiting with respect to the true spirit and scope of the present invention.

What is claimed is:

1. A chemopreventive composition for treating at least one of tumors, brain tumors, cancers, and growths in warm blooded animals sensitive to treatment comprising a composition having an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

2. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid in a free acid form.

3. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

4. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is dried parsley.

5. The composition as recited in claim 1 further comprising a pharmaceutically acceptable carrier or diluent.

6. The composition as recited in claim 1 wherein said carrier or diluent is distilled water.

7. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is a nutritional supplement containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

8. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is selected from the group of natural foods, processed foods, molds, plants, and vegetables containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

9. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid

and oxalate is selected from the group of beverages, liquids, and juices containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

10. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is selected from the group of additives containing at least one therapeutically effective form of at least one of oxalic acid and oxalate.

11. The composition as recited in claim 5, wherein said at least one carrier and diluent is a gel cap.

12. The composition as recited in claim 5, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

13. The composition as recited in claim 1, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

14. An anti-tumor agent to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising a mixture of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate with at least one of a pharmaceutically acceptable carrier and diluent.

15. The agent as recited in claim 14 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

16. The agent as recited in claim 14 wherein said pharmaceutically acceptable carrier or diluent is selected from the group of distilled water, heated water, pharmaceutically acceptable liquids, nutritional supplements, natural foods and processed foods.

17. The anti-tumor agent as recited in claim 14, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

18. The agent as recited in 14, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

19. A therapeutic composition in cream or ointment form for topical administration of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate, a solvent, and a base.

20. The therapeutic composition as recited in claim 19 wherein said solvent is at least one of distilled water, acetone, propylene glycol, and polysorbate, and said base is at least one of a cream, ointment, gel, lotion, spray, stick, and powder.

21. The therapeutic composition as recited in claim 19 wherein said solvent is at least one of distilled water, acetone, propylene glycol, and polysorbate and said base is at least one of a hydrophilic petrolatum, cream, ointment, gel, lotion, spray, stick, and powder.

22. The composition as recited in claim 19, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

23. In a pet food containing at least one of protein, carbohydrates, oils, vitamins, and minerals, to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment, the improvement comprising the addition of a therapeutically effective quantity of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

24. The pet food as recited in claim 23, wherein the effective quantity is about 1 mg to 3 g per pet.

25. In a method of manufacturing a dry process dog food to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment, the improvement comprising the steps of mixing a slurry of conventional dog food ingredients together with a dilute solution of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate in heated water to form at least one of an oxalic acid and oxalate containing slurry, forming the slurry into pellets and drying the pellets.

26. The method as recited in claim 25 wherein said oxalic acid is oxalic acid dihydrate and each pellet contains approximately 1 mg of oxalic acid so that one pound of dry dog food contains about 1 g of oxalic acid.

27. The method as recited in claim 25, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

28. An oral rinse or wash for treating at least one of tumors, cancers, and growths in warm blooded animals sensitive to treatment in the mouth area comprising a dilute solution of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said solution is adapted to be administered periodically in less than a lethal dosage.

29. The oral rinse or wash as recited in claim 28 comprising a dilute solution of oxalic acid dihydrate in distilled water.

30. The oral rinse as recited in claim 29, wherein said rinse contains a solution ratio of about 150 mg oxalic acid dihydrate in about 300 ml distilled water.

31. The oral rinse as recited in claim 28, wherein said rinse contains a solution ratio of about 150 mg oxalic acid dihydrate in about 300 ml distilled water.

32. A dietary supplement for treating a patient diagnosed with at least one of an active cancer, tumor, and growth sensitive to treatment comprising about 1 g to 6 g of at least one therapeutically effective form of at least one of oxalic acid and oxalate per day based on 70 kilograms of body weight and at least one of a pharmaceutically acceptable carrier and diluent.

33. The dietary supplement as recited in claim 32, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule,

lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

34. The dietary supplement as recited in claim **32**, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

35. A pharmaceutical composition to be administered orally to humans for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising a mixture of a non-toxic ingestible carrier and an effective amount of a therapeutically effective form of at least one of oxalic acid and oxalate.

36. The pharmaceutical composition as recited in claim **35**, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

37. The composition as recited in claim **35**, wherein said effective amount is about 50 mg to 6 g.

38. The pharmaceutical composition as recited in claim **35** wherein said composition is provided in a form selected from the group of pills, powders, granules, tablets, microcapsules, gel capsules, nutritional supplements, processed foods, liquids, drops, beverages, additives, and solutions.

39. In a pet treat containing at least one of protein, carbohydrates, and flavorings, to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment w, the improvement comprising the addition of microgram amounts of at least one therapeutically effective form of at least one of oxalic acid and oxalate, whereby the treats provide for the maintenance of good pet health.

40. The pet treat as recited in claim **39**, wherein the microgram amount provides for about 1 mg to 3 g per pet per day.

41. A chemopreventive composition for treating at least one tumor in a warm blooded animal sensitive to treatment comprising a composition including an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

42. The chemopreventive composition as recited in claim **41**, wherein the effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

43. A chemopreventive composition for treating at least one cancer in a warm blooded animal sensitive to treatment comprising a composition including an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

44. The chemopreventive composition as recited in claim **43**, wherein the effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

45. In a gel cap, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

46. The gel cap as recited in claim **45**, wherein the effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

47. In a pill, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

48. The pill as recited in claim **47**, wherein the effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

49. In a drug delivery system, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

50. The drug delivery system as recited in claim **49**, wherein the therapeutically effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

51. A method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate to said animal, wherein said dosage is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered periodically in less than a lethal dosage.

52. The method as recited in claim **51** wherein said composition is administered at least one of orally and sublingually.

53. The method as recited in claim **51** wherein said composition is administered by injection.

54. The method as recited in claim **51** wherein said composition is administered topically.

55. The method as recited in claim **51** wherein said composition is administered internally.

56. The method as recited in claim **51** wherein said composition is administered at least once a day at a dosage of at least one of 50 mg to 6 g for humans and 1 mg to 3 g for warm blooded animals other than humans.

57. The method as recited in claim **51** wherein said composition is administered at least once a day at a dosage of about 1 mg to 3 g for dogs and cats.

58. The method as recited in claim **51**, wherein said composition is administered by injection.

59. The method as recited in claim **51**, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

60. The method as recited in claim **51** further comprising the steps of reducing the intake of oxalic acid or oxalate blockers.

61. The method as recited in claim **60** wherein said blockers are selected from the group of citric acid, ascorbic acid, pyridoxine hydrochloride, calcium, alcohol, resins, clays, and combinations thereof.

62. The method as recited in claim 60 wherein said blockers are selected from the group of dairy products containing calcium, fruits, coconut, beverages containing alcohol, ascorbic acid or citric acid including adult beverages such as beer, wine, vodka, gin, and the like, fruit juice based beverages, soda pop or soft drinks containing ascorbic acid or citric acid, other sports drinks, beverages or refreshments containing calcium, ascorbic acid or citric acid, red meat or white meat of fowl including chicken, turkey, pheasant and the like containing pyridoxine hydrochloride, or other foods or beverages containing alcohol, citric acid, ascorbic acid, calcium or pyridoxine hydrochloride including breads or grains, and combinations thereof.

63. A method for treating at least one of cancers, tumors, and growths in warm blooded animals sensitive to treatment comprising the steps of adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

64. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid in a free acid form.

65. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is a nutritional supplement containing at least one form of at least one of oxalic acid and oxalate.

66. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

67. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is processed foods containing at least one form of at least one of oxalic acid and oxalate.

68. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is at least one of plants and vegetables containing at least one form of at least one of oxalic acid and oxalate.

69. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is at least one of beverages, liquids, and juices containing at least one form of at least one of oxalic acid and oxalate.

70. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is additives containing at least one form of at least one of oxalic acid and oxalate.

71. The method as recited in claim 63, wherein said effective amount is a daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

72. The method as recited in claim 63, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

73. The diet as recited in claim 63 further comprising the steps of reducing the intake of oxalic acid or oxalate blockers.

74. The diet as recited in claim 73 wherein said blockers are selected from the group of citric acid, ascorbic acid, pyridoxine hydrochloride, calcium, alcohol, resins, clays, and combinations thereof.

75. The diet as recited in claim 73 wherein said blockers are selected from the group of dairy products containing calcium, fruits, coconut, beverages containing alcohol, ascorbic acid, or citric acid including adult beverages such as beer, wine, vodka, gin, and the like, fruit juice based beverages, soda pop or soft drinks containing ascorbic acid or citric acid, other sports drinks, beverages or refreshments containing calcium, ascorbic acid or citric acid, red meat or white meat of fowl including chicken, turkey, pheasant, and the like containing pyridoxine hydrochloride, or other foods or beverages containing alcohol, ascorbic acid, citric acid, calcium or pyridoxine hydrochloride including breads or grains, and combinations thereof.

76. A method of treating warm-blooded animals afflicted with tumor cells sensitive to an effective amount of at least one of an oxalic acid and oxalate compound comprising the steps of periodically administering to the animal an oncolytic amount of at least one therapeutically effective oxalic acid and oxalate compound.

77. The method as recited in claim 76, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

78. The method as recited in claim 76, wherein said compound is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

79. A method of treating brain tumors in warm blooded animals sensitive to treatment comprising the steps of ingesting or administering a therapeutically effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

80. The method as recited in claim 79 wherein the therapeutically effective form of at least one of oxalic acid and oxalate is dried chopped parsley.

81. The method as recited in claim 79, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

82. The method as recited in claim 79, wherein said effective form of at least one of oxalic acid and oxalate is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

83. A method for treating at least one of tumors, growths, and cancers in warm-blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of the composition of claim 1 and adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

84. The method as recited in claim 83, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid,

red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

85. The method as recited in claim 83, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

86. A method for treating cancer in warm blooded animals sensitive to treatment, comprising the steps of:

periodically administering a therapeutically effective dosage of at least one therapeutically effective form of at least one of oxalic acid and oxalate to said animal, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said solution is adapted to be administered periodically in less than a lethal dosage.

87. The method as recited in claim 86, wherein said composition is administered at least one of orally and sublingually.

88. The method as recited in claim 86, wherein said composition is administered by injection.

89. The method as recited in claim 86, wherein said composition is administered topically.

90. The method as recited in claim 86, wherein said composition is administered internally.

91. The method as recited in claim 86, wherein said composition is administered at least once a day at a dosage of at least one of 50 mg to 6 g for humans and 1 mg to 3 g for warm blooded animals other than humans.

92. The method as recited in claim 86, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

93. A method for treating at least one tumor in a warm blooded animal sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage to said animal.

94. The method as recited in claim 93, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

95. The method as recited in claim 93, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

96. A method for treating at least one cancer in a warm blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage to said animal.

97. The method as recited in claim 96, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

98. The method as recited in claim 96, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

99. A treatment regimen for treating tumors, cancers, growths, and neoplasia in warm blooded animals sensitive to treatment comprising the steps of reducing or eliminating the ingestion or administration of oxalic acid or oxalate blockers, administering or ingesting high dosages of at least one of oxalic acid and oxalate to raise the blood or urine oxalic acid or oxalate level above normal, and, after cleansing the blood of tumor, cancer or abnormal cells, administering or ingesting a more moderate level of at least one of oxalic acid and oxalate to maintain a normal blood or urine oxalic acid or oxalate level.

100. The regimen as recited in claim 99 further comprising the steps of increasing the administration or ingestion of oxalic acid or oxalate enhancers.

101. A chemopreventive composition for treating at least one of tumors, brain tumors, cancers, and growths in humans sensitive to treatment comprising a composition having an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered on a periodic basis in less than a lethal dosage.

102. A method for treating at least one of tumors, growths, and cancers in humans sensitive to treatment comprising the steps of periodically administering at therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate, wherein said dosage is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered periodically in less than a lethal dosage.

103. The method as recited in claim 102 wherein said composition is administered at least one a day at a dosage of about 50 mg to 6 g.

104. A method for treating at least one of cancers, tumors, and growths in humans sensitive to treatment comprising the steps of adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

* * * * *

1. Simple ointment, oleaginous:

white wax 25 g

yellow wax 25 g

petrolatum 950 g

The proportion of wax can be varied to obtain a suitable consistency of the ointment.

2. Hydrophilic Petrolatum (this preparation will absorb large amount of water of aqueous solutions of active ingredient, form water-in-oil type emulsions):

Cholesterol 30 g

stearyl alcohol 30 g

white wax 80 g

white petrolatum 860 g

3. Lanolin, Hydrous or anhydrous wool fat.

4. Washable Hydrophilic Ointment (oil-in-water emulsion):

methylparben 0.025 g

propylparben 0.015 g

sodium lauryl sulfate 10 g

propylene glycol 120 g

stearyl alcohol 250 g

white petrolatum 250 g

purified water 370 g

5. Polyethylene Glycol Ointment:

polyethylene glycol 3350 350 g

polyethylene glycol 400 600 g

stearyl alcohol 50 g

6. Absorbent dusting powders

Magnesium stearate 100 g

7. Absorbent dusting powders:

Magnesium stearate 50 g

Boric Acid 50 g

8. Absorbent/adsorbent Delivery Particles:

Mixtures of porous and non-porous micro beads.

The formulations listed above are for illustrative purposes only. Moreover, the treating protocol used to treat or prevent diaper dermatitis can include one or more of the compositions of the present invention as well as one or more of the formulations set forth above. Of course, to the vehicles shown above may be added the enzyme inhibitors or inactivators and/or the additional therapeutically effective adjuvants as set forth herein to address the effects of diaper dermatitis.

The compositions or formulations of this invention are generally prepared by mixing an effective amount of an enzyme inhibiting system alone or in conjunction with an adjuvant system into a desired vehicle under conditions of time, temperature and pressure to facilitate the formation of a substantially homogeneous composition. Generally, the mixing time is between about 0.1 minutes and about 1 hour, preferably between about 0.2 minutes and about 40 minutes and particularly between about 1 minute and about 30 minutes. Generally, the temperature is between about 50° F. and about 300° F., preferably between about 60° F. and about 200° F., and particularly about room temperature. Generally, the pressure is between about 0.5 atmosphere and about 25 atmospheres, preferably between about 1 atmosphere and about 5 atmospheres, and particularly at about standard atmospheric pressure. The mixing can be carried out in a traditional open mixer, a mixing extruder, a blender or any other mixing apparatus well-known in the art. Of course, the mixing time, mixing temperature and mixing pressure can be adjusted to the particular equipment being used provided that the temperature does not exceed a decomposition temperature for the inhibitors or any other component of the composition.

One ointment formulation including an enzyme inactivating system of the present invention for direct application to skin follows:

8 g of cholestyramine (a weak anion resin)

50 g of Euserin a water based glycerin carrier.

200 mg of tetrahydrolipstatin

500 mg of trypsin-chymotrypsin inhibitor from Sigma Chemicals.

All references cited herein are incorporated by reference. While this invention has been described fully and completely, it should be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.

I claim:

1. A composition for treating enzyme induced tissue damage in an acidic environment comprising an effective amount of a treating system selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease, substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, wherein the composition neutralizes acid activity.

2. The composition of claim 1, wherein the enzyme inhibitor system comprises a lipase inhibitor.

3. The composition of claim 2, wherein the lipase inhibitor is selected from the group consisting of esterastin, lipstatin, valilactone, tetrahydrolipstatin, panclicin, ebelactone, ajoene and mixtures or combinations thereof.

4. The composition of claim 1, wherein the enzyme inhibitor system comprises a protease inhibitor.

5. The composition of claim 4, wherein the protease inhibitor comprises a trypsin-chymotrypsin inhibitor.

6. The composition of claim 1, wherein the enzyme inhibitor system comprises a lipase inhibitor and a protease inhibitor.

7. The composition of claim 6, wherein the lipase inhibitor is esterastin, lipstatin, valilactone, tetrahydrolipstatin, panclicin, ebelactone, ajoene or mixtures or combinations thereof and the protease inhibitor is a trypsin-chymotrypsin inhibitor.

8. The composition of claim 1, further comprises effective concentrations of therapeutic adjuvants, where the therapeutic adjuvants comprises anti-inflammatory agents, antimicrobial agents, antiboitic agents, hemostatic agents, anaesthetic agents, anti-fungal agents or mixtures or combinations thereof.

9. The composition of claim 1, further comprises a performance indicator to monitor the integrity of the enzyme inhibitor system.

10. The composition of claim 1, further comprising a carrier.

11. A method for treating enzyme damage to tissue in an acidic environment by:

administering to tissue of a subject an effective amount of an enzyme treating system, where the treating system is

selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, where the system neutralizes acid activity.

12. A method for treating enzyme damage to tissue in an acidic environment by:

periodically administering to a tissue of a subject an effective amount of an enzyme treating system, where the treating system is selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, where the system neutralizes acid activity.

13. A method for preparing a composition for treating enzyme damage to tissue in an acidic environment by:

mixing an effective amount of an enzyme treating system selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system and combinations thereof into a vehicle, where the system neutralizes acid activity.

* * * * *

invention to utilize a unit dosage form which will contain from about 250 mg. to about 800 mg. of 5-methyl-3-sulfanilamidoisoxazole or an equivalent amount of a salt thereof and from about 12.5 mg. to about 160 mg. of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or an equivalent amount of a salt thereof. The frequency with which any such unit dosage form will be administered to a warm-blooded animal will vary, depending upon the quantity of medicament present therein and the needs and requirements of the warm-blooded animal. Under ordinary circumstances, however, about a total of 60 mg./kg. of 5-methyl-3-sulfanilamidoisoxazole and about a total of 8 mg./kg. of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, in combination, can be administered daily in several doses.

As hereinabove discussed, detailed description is made with reference to unit dosages whether in intravenous or oral form, the frequency and dosage levels are best related with regard to the effectiveness in treating a fibrosarcoma (SAD_2) tumor in terms of component levels in the plasma of the warm-blooded animals being treated of the composition selected from the group consisting of 5-methyl-3-sulfanilamidoisoxazole, a salt of 5-methyl-3-sulfanilamidoisoxazole with a pharmaceutically acceptable base, 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, a salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine with a pharmaceutically acceptable acid and mixtures thereof. Generally, it is preferably desired to maintaini in the plasma of the warm-blooded animal a component level of the 5-methyl-3-sulfanilamidoisoxazole or an equivalent amount of the salt thereof of from about 80 to 160, preferably about 110 μg./cc. and/or a component level of the 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or an equivalent amount of the salt thereof of from about 5 to 15, preferably 10 μg./cc.

This invention relates to the invention described in copending applications (Ser. Nos. 655,079; 655,227; 655,145; and 655,080; now U.S. Pat. Nos. 4,632,920; 4,698,335; 4,632,919; and 4,661,478, respectively filed on even date herewith, the teachings of which are incorporated) by reference herein.

The foregoing, notwithstanding, it should be fully understood that the dosages set forth herein are exemplary only and they do not, to any extent, limit the scope or practice of the present invention. As indicated hereinbefore, the combination of this invention has unexpectedly been found to be particularly useful for its effects in the treatment of a fibrosarcoma (SAD_2) tumor in warm-blooded animals.

The invention will be understood better by reference to the following examples which are given for illustration purposes and are not meant to limit the invention.

EXAMPLES

CDF_1 mice (23 +2 gm.) were implanted with a fibrosarcoma (SAD_2) subcutaneously. One week later all mice received pentobarbital sodium anesthesia followed by either 0.1 cc. Injectable Composition (Trimethoprim 16 mg./cc., Sulfamethoxazole 80 mg./cc., Dosage: 320 mg./kgS, 64 mg./kgT) or an equal volume of saline. One hour later all mice received 400 rads (8MeV Photon) to the tumor.

TABLE

Two Week Survivals			
Saline	2/20	20%	$p < .05$
I.C.	5/11	46%	

The two week survival time of recipients of the Injectable Composition was significantly greater than that of the control and suggest beneficial effects in neoplasia of such Injectable Composition.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

What is claimed:

1. A process for treating a warm-blooded animal having a fibrosarcoma (SAD_2) tumor which comprises administering to said animal
(a) 400 rads of radiation,
(b) from about 1 to about 30 parts selected from the group of 5-methyl-3-sulfanilamidoisoxazole and a alkali metal salt of 5-methyl-3-sulfanilamidosoxazole with a pharmaceutically acceptable base and,
(c) from about 30 to about 1 part selected from the group of 2, 4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine and a salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine with a pharmaceutically acceptable acid,
said compounds (b) and (c) being administered in an amount effective to lengthen the survival of said animal having a tumor.

2. The process as defined in claim 1 wherein the effective amount of the compounds administered comprise from about 250 mg to about 800 mg of 5-methyl-3-sulfanilamidoisoxazole or an alkali metal salt thereof with a pharmaceutically acceptable base and from about 12.5 mg. to about 160 mg. of 2,4-di-amino-5 (3,4,5-trimethoxy benzyl) pyrimidine or a salt thereof with a pharmaceutically acceptable acid.

3. The process of claim 1 wherein the compounds are administered intravenously.

4. The process of claim 1 wherein the compounds are administered orally.

5. The process of claim 1 wherein the pharmaceutically acceptable base with which the alkali metal salt of 5-methyl-3-sulfanilamidoisoxazole is formed is selected from the group consisting of sodium hydroxide and potassium hydroxide.

6. The process of claim 1 wherein the pharmaceutically acceptable acid with which the salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine is formed is a mineral acid or an organic acid.

7. The process of claim 6 wherein the mineral acid is selected from the group consisting of hydrochloric acid and sulfuric acid.

8. The process of claim 6 wherein the organic acid is selected from the group consisting of acetic acid, citric acid, lactic acid, maleic acid and salicyclic acid.

9. The process of claim 1 wherein the compounds are administered individually.

10. The process of claim 1 wherein the ratio of the amount administered of 5-methyl-3-sulfanilamidoisoxazole or an equivalent salt and the amount administered of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine is between about 5 and about 15 to 1.

11. The process of administering the compounds of claim 1 wherein up to about 160 mg/kg of 5-methyl-3-

sulfanilamidoisoxazole and up to about 8 mg/kg of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine are administered daily to said warm-blooded animal.

12. The process of claim **11** wherein the daily administration of the compounds is in several doses.

13. The process of claim **1** wherein the radiation is administered subsequent to the administration of the compounds.

14. A therapeutic method for treatment of a fibrosarcoma (SAD_2) tumor in a warm-blooded animal which comprises periodically administering to said warm-blooded animal 400 rads of radiation and a therapeutic composition which comprises a therapeutically effective amount of an antineoplastic/antimetastatic compound selected from the group consisting of 5-methyl-3-sulfanilamidoisoxazole or an alkali metal salt thereof with a pharmaceutically acceptable base sufficient to maintain a blood plasma concentration of said compound between about 80 and about 160 micrograms/cc.

15. The process as defined in claim **14** wherein a composition concentration is maintained at about 110 μg./cc.

16. A therapeutic method for treatment of a fibrosarcoma (SAD_2) tumor in a warm-blooded animal which comprises periodically administering to said warm-blooded animal 400 rads of radiation and a therapeutically effected amount of an antineoplastic/antimetastatic composition selected from the group consisting of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or a salt thereof with a pharmaceutically acceptable acid sufficient to maintain a blood plasma concentration of said compound between about 5 and about 15 micrograms/cc.

17. The process as defined in claim **16** wherein a composition concentration is maintained at about 10 μg./cc.

* * * * *

QUEST Volume 10, Number 2, MARCH/APRIL 2003

Pompe's Disease

A Killer Yields to Modern Medicine

by *Dan Stimson*

You may never have heard of Pompe's disease. It affects just 5,000 to 10,000 people in the United States, making it exceedingly rare and of little interest to the general public. But what it lacks in notoriety, it makes up for in personal devastation to those who have it.

Robert Elmore nuzzles his son Grante ("Nikko"), who has lived twice as long as expected thanks to enzyme replacement therapy provided in a clinical trial.
Photos by Amy Snyder

Pompe's (also known as acid maltase deficiency) is caused by a genetic deficiency of an enzyme that breaks down glycogen (stored sugar) inside muscle cells. In its severest form, it strikes during infancy, weakening the heart and the voluntary muscles, including those that control breathing. The disease can also manifest during childhood or adulthood, causing significant muscle weakness and respiratory problems.

Children and adults with the disease usually have a shortened life span, and most infants with the disease aren't expected to live beyond 1 year of age.

But these grim prognoses could soon change, thanks to research led by Yuan-Tsong Chen, professor and chief of Medical Genetics in the Department of Pediatrics at Duke University in Durham, N.C., and director of the Institute of Biomedical Sciences, Academia Sinica, Taiwan. Through basic research supported by MDA and clinical trials supported by the biotech company Genzyme, Chen and his team at Duke have developed a way to supply the missing enzyme to people with Pompe's disease.

In two trials, one completed in 2000 and the other last year, 11 babies have received this experimental treatment — called enzyme replacement therapy — and some are now healthy, walking toddlers.

A Faulty Enzyme, Failing Muscles

With currently available treatment, "There's not much we can do for babies with Pompe's disease," Chen says.

Within weeks or months of birth, an infant with the disease can become too weak to suckle or breathe on its own. The muscular walls of the heart become enlarged, shrinking the heart's inner chambers and reducing its pumping capacity, a condition known as *hypertrophic cardiomyopathy*. Most babies with the disease die from cardiac and respiratory failure within three to four months of diagnosis, Chen says.

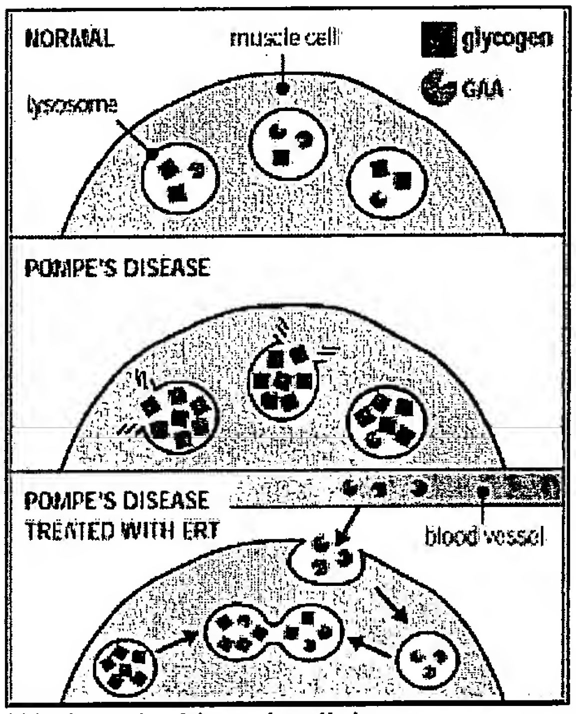
This picture of infant-onset Pompe's disease has changed little since it was first described by Dutch pathologist Joannes Pompe in the early 1930s. While studying at the University of Amsterdam, Pompe was asked to do an autopsy on a 7-month-old girl who had been admitted to the university hospital with difficulty breathing and had died three days later, apparently of pneumonia. Expecting to see her lungs filled with fluid, he was surprised to find that her heart had swollen to more than three times its normal size and the cells within it were filled with clumps of debris, which turned out to be glycogen.

It wasn't until the 1960s that other researchers discovered the underlying basis of Pompe's disease — a deficiency of the enzyme *acid alpha-glucosidase (GAA)*, also called *acid maltase*. The deficiency can now be detected by blood tests that probe for GAA activity or for mutations in the gene encoding GAA, located on chromosome 17. It's estimated that one in 85 to 100 people carries a mutation in a single copy of the gene; it takes mutations in both copies, one inherited from each parent, to cause the disease.

The severity of mutations in the GAA gene — that is, how much they alter the enzyme — determines, at least in part, the severity of the disease.

Mutations that destroy the protein cause infant-onset Pompe's disease, while mutations that leave some GAA intact tend to cause juvenile- and adult-onset forms of the disease.

The later-onset forms of Pompe's are primarily "muscle diseases," Chen says. Cardio-myopathy is mild in the juvenile form, and usually absent from the adult form. In children, the most common first symptom is delayed motor development; in adults, it's difficulty walking. For both late-onset forms, respiratory weakness can be severe and often requires mechanical ventilation, Chen says.



Working inside subcellular compartments called lysosomes, the enzyme acid alpha-glucosidase (GAA) breaks down glycogen (top). In Pompe's disease, a deficiency of GAA causes glycogen to accumulate and rupture lysosomes (middle). In enzyme replacement therapy (ERT), intravenously injected GAA is taken up by lysosomes that have fused with the cell's outer surface, eventually making its way to glycogen-filled lysosomes (bottom).

The Lysosome Connection

Chen has been studying glycogen storage disease, a category of diseases that includes Pompe's, for more than 20 years. (Pompe's disease is sometimes called *glycogen storage disease type 2* or *acid maltase deficiency*; it's one of 10 *metabolic diseases of muscle* covered by MDA's program.)

Early in his career, he recognized that Pompe's disease was going to be a tough nut to crack.

In most glycogen storage diseases, inadequate breakdown of glycogen leads to hypoglycemia, a drop in blood sugar levels that drains the body of energy. In these diseases, supplementing the diet with complex sugars like cornstarch can help maintain blood sugar levels and control symptoms.

But the symptoms of Pompe's disease aren't related to hypoglycemia; instead, they're caused by the accumulation of glycogen itself. GAA is one of many enzymes found in *lysosomes*, compartments inside cells that "clean house" by trapping and degrading glycogen and other chemicals. Without GAA, glycogen builds up inside lysosomes and ruptures them, an effect that's especially damaging to muscle, which naturally makes large amounts of the energy-rich substance. (This makes Pompe's disease a glycogen storage disease *and* a lysosomal storage disease, a category that includes Tay-Sachs and Niemann-Pick diseases.)

Chen has thus focused much of his research on how to deliver GAA to the lysosomes of people with Pompe's disease. This is a tall order, one that might seem to parallel efforts at gene therapy and stem cell therapy for

muscle disease, which haven't yet shown success in the clinic.

But Chen has been able to exploit a key feature of lysosomes: In their business of "housecleaning," they fuse with the outer surface of the cell, allowing them to release their contents and take up substances from the outside. Because lysosomes take in other substances, Chen and others reasoned that intravenously delivered GAA might make its way into the lysosomes of muscle cells.

Yuan-Tsong Chen.
Photos by Cramer Gallimore

Building a Better Enzyme

Enzyme replacement therapy for Pompe's disease wasn't his idea, Chen acknowledges. In clinical trials in the 1970s, patients with the disease were given injections of GAA isolated from human placenta, but the treatment failed.

Later, Chen says, "We learned that in order for the enzyme to work, you need to make a special form of it that can be taken up by the cells in [voluntary] muscles and in the heart. The second critical issue is how to make sufficient quantities of the enzyme for a clinical trial."

By the 1980s, scientists discovered that for efficient uptake by lysosomes in muscle cells, GAA and other enzymes must have a chemical "tag," called *mannose-6-phosphate (M6P)*. Human placenta had been a plentiful source of GAA, but it produces a version of the enzyme that has very little M6P.

In the 1990s, with advances in molecular biology and funds from MDA, Chen was able to engineer an M6P-laden version of the enzyme — called *recombinant human GAA (rhGAA)* — using a line of cells (*CHO cells*) to produce it in large amounts.

Armed with this new enzyme, Chen formed a collaboration with researchers from Tokyo, who were studying a strain of Japanese quail rendered flightless by naturally occurring Pompe's disease. After three weeks of injections with rhGAA, the birds could fly.

Chen and his team were ready to test rhGAA in babies with Pompe's disease, but first, they needed help from the biotech industry.

"In order to test the therapy in humans," Chen explains, "we needed to make the enzyme in a GMP [good manufacturing

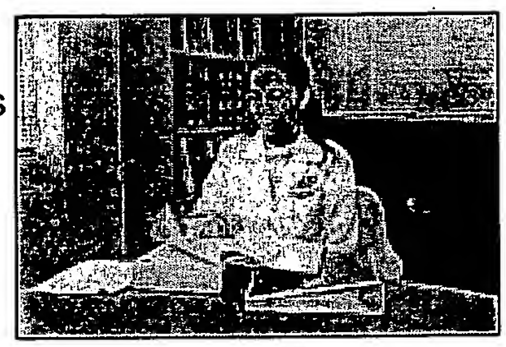
Andy Amalfitano

practice] facility, we needed to have every single step documented, and we needed large bioreactors" — incubation chambers for growing the CHO cells that produce rhGAA. "These are things we're not able to do in an academic research lab."

A Lifesaving Treatment

Working first with Synpac, a pharmaceutical company based in Taiwan, and later with Genzyme, a Cambridge, Mass.-based company with a longstanding interest in lysosomal storage diseases, Chen began his first trial of enzyme replacement therapy for Pompe's disease in 1999. The results were published in March 2001 in the journal Genetics in Medicine.

The three babies in the trial, who ranged from 2 months to 4 months old at its inception, had once been expected to die — but all of them are still alive. After about a year of twice-weekly intravenous infusions with rhGAA, all experienced significant reductions in heart size and improvements in cardiac function. Genzyme has continued to supply them with the treatment.



Priya Kishnani

One baby has become "an essentially normal 3-year-old boy," able to walk and to breathe on his own, Chen says. The other two, now 3 and 4 years old, require mechanical ventilation and haven't developed normal motor skills, but they have normal cardiac function, he says.

In 2001, Genzyme and Duke scientists launched a second trial involving eight babies, ranging from 3 months to 14 months old. Five of the infants were studied at Duke and the others were studied at sites in Europe. Details of the results await publication, but Priya Kishnani, the trial's lead investigator at Duke, presented some of her data at a scientific meeting in Dublin in September.

According to her report, all of the babies experienced significant reductions in heart size, two died from complications unrelated to the enzyme, and the remaining six were still alive after about a year of treatment. (For more about one of these toddlers, see "A Time to Celebrate.")

"It's such a fruitful experience to go from a diagnostic approach to a treatment approach for a disease that's considered lethal," Kishnani says. "By no means is this a cure; we don't know the long-term benefits or side effects of the treatment. But there's nothing else out there right now to change the natural course of this devastating disease."

What the Future Holds

This year, Genzyme and the Duke team, led by Kishnani, will begin two larger trials of enzyme replacement therapy for Pompe's disease.

The trials are a final step toward getting the treatment approved by the U.S. Food and Drug Administration, Chen says. One, already under way, is enrolling toddlers between 6 months and 3 years old and the other will enroll babies less than 6 months old. (For more information, contact Genzyme Medical Information at [800] 745-4447.) Each trial will recruit up to 16 patients and will test a different version of rhGAA than that used in the previous trials.

Genzyme, which has made Pompe's disease its largest research and development effort since its founding 21 years ago, now has an arsenal of rhGAA types. The company began testing enzyme replacement therapy for Pompe's disease in 1998, through a joint venture with Pharming, a Dutch biotech company. Scientists from the two companies genetically engineered rabbits to produce rhGAA in their milk, and had begun testing this "transgenic" rhGAA in patients with the infantile and juvenile forms of Pompe's disease. In 2001, Pharming went into receivership and Genzyme acquired the rights to the transgenic rhGAA.

Genzyme acquired another type of rhGAA, made in CHO cells like Chen's, when it bought the Princeton, N.J., company Novazyme Pharmaceuticals.

Recently, Genzyme has developed a fourth version of rhGAA with "improved scalability," meaning it can be produced in larger quantities than previous versions. This is the enzyme that Duke researchers will test in upcoming trials; once a sufficient amount of the enzyme is available, they hope to test it in adults with Pompe's disease.

Looking to the more distant future, scientists at Genzyme and Duke are also investigating gene therapy for Pompe's disease. One potential benefit of this approach is "a decreased need for frequent infusions of the enzyme. You could envision a gene therapy treatment that would only be required yearly," says Andy Amalfitano, a co-investigator in the enzyme replacement therapy trials.

In fact, Amalfitano says, "Pompe's disease may be one of the best diseases to consider treating by gene therapy... because we have an opportunity to treat every muscle in the body without inserting the [GAA] gene into every muscle." A virus could be used to deliver GAA to the liver, which could then release the enzyme into the bloodstream, Amalfi-tano explains. In MDA-funded experiments at Duke, he's used this approach to restore GAA activity to the muscles of mice and quail with Pompe's disease.

Editor's Note: Until now, MDA and Genzyme have made independent efforts to support the development of enzyme replacement therapy for Pompe's disease. In November, MDA and Genzyme staff met at MDA's National Headquarters in Tucson, Ariz., and discussed plans to collaborate on future research.

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EXHIBIT C

ORIGINAL PAPER

Arnold J.J. Reuser · Hannerieke Van den Hout
Agnes G.A. Bijvoet · Marian A. Kroos
Martin P. Verbeet · Ans T. Van der Ploeg

Enzyme therapy for Pompe disease: from science to industrial enterprise

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Abstract Pompe disease or glycogen storage disease type II (OMIM 232300) is a metabolic myopathy with a broad clinical spectrum. Generalised muscle weakness combined with cardiomegaly presents within the first 3 months after birth, if the lysosomal α-glucosidase (AGLU) deficiency is complete. Residual enzyme activity prevents cardiac involvement and delays onset of muscle weakness. Enzyme therapy, by intravenous administration of acid AGLU, aims to supplement the missing enzyme activity. At the SHS symposium on Glycogen Storage Diseases Type I and II, in Fulda, two interim accounts were given of studies on the efficacy of enzyme therapy for Pompe disease; one with recombinant human acid AGLU produced in Chinese hamster ovary cells and the other with the same enzyme produced in the milk of transgenic rabbits. *Conclusion:* this review focuses on the latter study, discusses the scientific, technological and commercial aspects of the enterprise, and addresses the prospects and challenges of enzyme therapy for Pompe disease.

Keywords Acid maltase deficiency · Enzyme therapy · Glycogenosis · Lysosomal storage disease · Transgene technology

Abbreviations *AGLU* α-glucosidase · *CHO* Chinese hamster ovary · *CRIM* cross-reactive immunological material · *M6P* mannose-6-phosphate

A.J.J. Reuser (✉) · A.G.A. Bijvoet · M.A. Kroos
Department of Clinical Genetics,
Erasmus University Rotterdam, PO Box 1738,
3000 DR Rotterdam, The Netherlands
E-mail: reuser@ikg.fgg.eur.nl
Tel.: +31-10-4087153
Fax: +31-10-4089489

H. Van den Hout · A.T. Van der Ploeg
Department of Paediatrics, Division of Metabolic Diseases,
Sophia Children's Hospital, Rotterdam, The Netherlands

M.P. Verbeet
Institute of Chemistry, Leiden University,
Leiden, The Netherlands

Enzyme therapy in historic perspective

Over the past 35 years we have learned what it takes to bring enzyme therapy for lysosomal storage disorders into practice. The concept of enzyme therapy is built on the key function of lysosomes in cell and tissue renewal. Macromolecular compounds, even whole cell organelles like mitochondria, are recycled by the lysosomal system (Fig. 1). Materials derived from the intra-cellular space are sequestered by membranes and delivered through fusion of newly formed autophagic vacuoles with lysosomes. Extra-cellular materials are taken up by bulk or receptor mediated endocytosis and are delivered by fusion of endosomes with lysosomes. Once inside lysosomes, the material is degraded by one or a combination of several lysosomal hydrolases. Some of these hydrolases are assisted by activator proteins [23].

The role of lysosomes in cellular pathology became evident in 1963 with the discovery of acid α-glucosidase (AGLU) deficiency as primary defect in Pompe disease or glycogen storage disease type II [13]. Knowing the function of lysosomes, it was envisaged that patients with lysosomal storage disorders could be treated by administration of the missing enzyme that would find its way to the lysosomes via endocytosis (Fig. 1) [9]. Expectations were high and regulations concerning the performance of clinical studies were less strict in those days than they are at present. The first attempt at enzyme therapy dates from 1964 and involved treatment of a patient with Pompe disease with acid AGLU from the fungus *Aspergillus niger* [5]. Similar experiments followed using enzyme preparations from various other sources [30]. A slight increase of acid AGLU activity in liver was obtained in some of these studies upon intravenous infusion [19], but not in muscle. Reduction of liver lysosomal glycogen only was obtained with high enzyme doses over long periods of time. In all instances, lack of ultimate effect and occurrence of serious side-effects terminated the treatment.

In the period 1965–1980, numerous reports were published about enzyme replacement therapy in several of the lysosomal storage diseases, but most results were basically negative [30]. Some important facts became evident. Enzyme preparations from non-human sources are antigenic. The blood brain barrier cannot be crossed, so that patients with central nervous system involvement cannot be treated for mental and motor dysfunction.

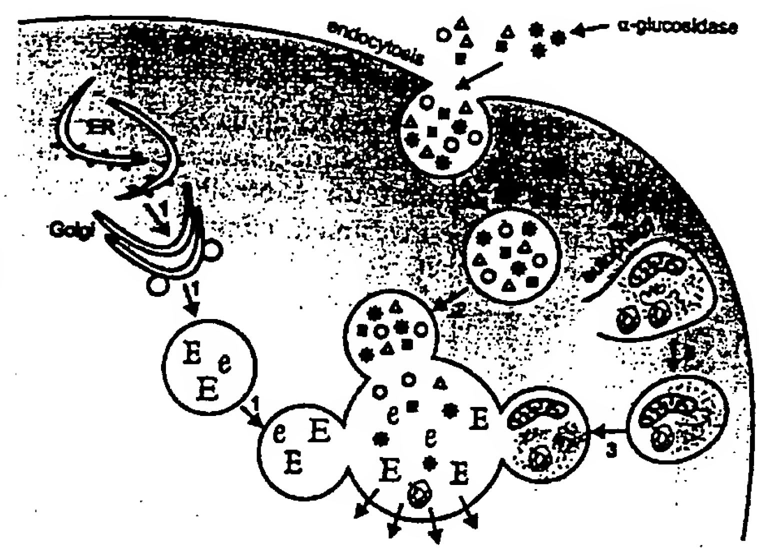


Fig. 1. Enzyme replacement therapy in lysosomal storage diseases. Newly synthesised lysosomal enzymes enter the lumen of the endoplasmic reticulum co-translationally. They are glycosylated, folded, and equipped with the M6P recognition marker as lysosomal targeting signal (route 1). Inside lysosomes, they catalyse the digestion of biological compounds that are delivered by endocytosis (extracellular) (route 2) and autophagy (intracellular) (route 3). Inherited deficiency of lysosomal enzymes leads to lysosomal storage diseases. Lysosomal enzymes administered to cells are taken up via bulk (inefficient) and receptor mediated (efficient) endocytosis and are delivered to the lysosomes where they can supplement the missing enzyme. (*E* enzymes, *ER* endoplasmic reticulum)

Advanced technology is required to secure substantial enzyme supplies over long periods. Around 1980, the focus shifted from enzyme therapy to bone marrow transplantation [15].

A few people pursued the original concept and gave enzyme therapy a second and better chance by applying newly acquired knowledge on receptor mediated endocytosis. Ashwell and Morell [2] were among the pioneers to demonstrate the role of the asialoglycoprotein receptor (galactose) in uptake of glycoproteins by hepatocytes. The mannose receptor was shown to facilitate uptake of proteins with mannose-terminating carbohydrate side chains by Kupffer cells and macrophages of spleen and bone marrow [29], and fibroblasts were found to exchange lysosomal proteins via the mannose 6-phosphate (M6P) receptor [14, 21]. Brady and his group [3] were the first to demonstrate the potential of receptor mediated enzyme therapy for lysosomal storage disease in a patient with Gaucher disease. The glucocerebrosidase used in the study was purified from human placental tissue. The complex carbohydrate side chains were trimmed by sequential action of neuraminidase, galactosidase and N-acetyl-glucosaminidase in order to expose mannose residues and thereby target the enzyme to Kupffer cells and macrophages in spleen and bone marrow; the major sites of lysosomal glycolipid storage in Gaucher disease. The study turned out successful enough to convince scientists, patients and industry of the feasibility of receptor mediated enzyme therapy for Gaucher disease [4].

Enzyme therapy for Pompe disease

We have worked along parallel lines to investigate the feasibility of receptor mediated enzyme therapy for Pompe disease. Table 1 lists the critical events by date.

Table 1. Enzyme therapy for Pompe disease from science to industrial enterprise

Event	Production	
	Milk enzyme	CHO enzyme
Enzyme therapy: proof of principle	[35]	
AGLU cDNA cloning	[16]	
	[24]	
AGLU gene cloning	[17]	
	[25]	
Production	[6]	[11]
Testing in animals	[7]	[36]
	[7]	[36]
	[8]	[22]
		[7]
Industry enters the scene	Pharming (1991)	Synpac (1994)
	Genzyme (1998)	KDL BioTech (2000)
		Genzyme (2000)
		Pharming (2000)
		Novazyme (2000)
Orphan drug designation	1996	1997
Phase I trial	1998	-
Phase II trial	1999	-
Phase I/II trial	-	1999
Phase II trial data	[31]	[1]
Phase II/III trial	Not planned	May 2001

The target tissues in Pompe disease are muscle and heart. Patients have the same acid AGLU deficiency in all tissues, but the lysosomal glycogen accumulation and the symptomatology are largely restricted to skeletal muscle when the residual enzyme activity is 5%–25% of the normal range. At lower activity levels, heart and other tissues become increasingly involved. There is typically a clinical spectrum from early onset very severe to late onset mild disease. Affected infants have cardiomegaly around birth. They present as floppy babies and die usually before 1 year of age due to cardiorespiratory failure. Onset of symptoms is delayed, and cardiomegaly prevented by low levels of residual AGLU activity. In extreme cases, the skeletal muscle weakness may remain obscure until the sixth decade [27].

Our initial studies on the feasibility of enzyme replacement therapy for Pompe disease were directed towards establishing the presence of M6P receptors on cardiomyocytes and skeletal muscle cells and testing whether these could be employed to facilitate uptake of acid AGLU [26]. To this end, muscle biopsies of patients were dissociated with collagenase and trypsin, and myoblasts (satellite cells) were taken into culture. It turned out no problem to correct the lysosomal glycogen storage in these cells by addition of M6P-containing acid AGLU to the culture medium [32, 33, 34]. Herewith, the first requirements were fulfilled, but the experimental set-up does not mimic the reality in detail. When enzyme therapy is performed via intravenous infusion, the muscle cells do not come into direct contact with the enzyme like they do in tissue culture. The endothelial barrier of the capillaries needs to be crossed and the interstitial connective tissue (endomysium) needs to be passed. In this respect, the situation in Pompe disease is far different from that in Gaucher disease where there are no barriers between the Kupffer cells and the enzyme in the circulating blood. In Fabry disease the situation also is favourable compared to Pompe disease because the endothelial cells are a primary target. Nevertheless, we did obtain uptake of acid AGLU in muscle and heart of mice after intravenous administration of M6P-containing AGLU from bovine testis. The uptake of AGLU without M6P was less [35].

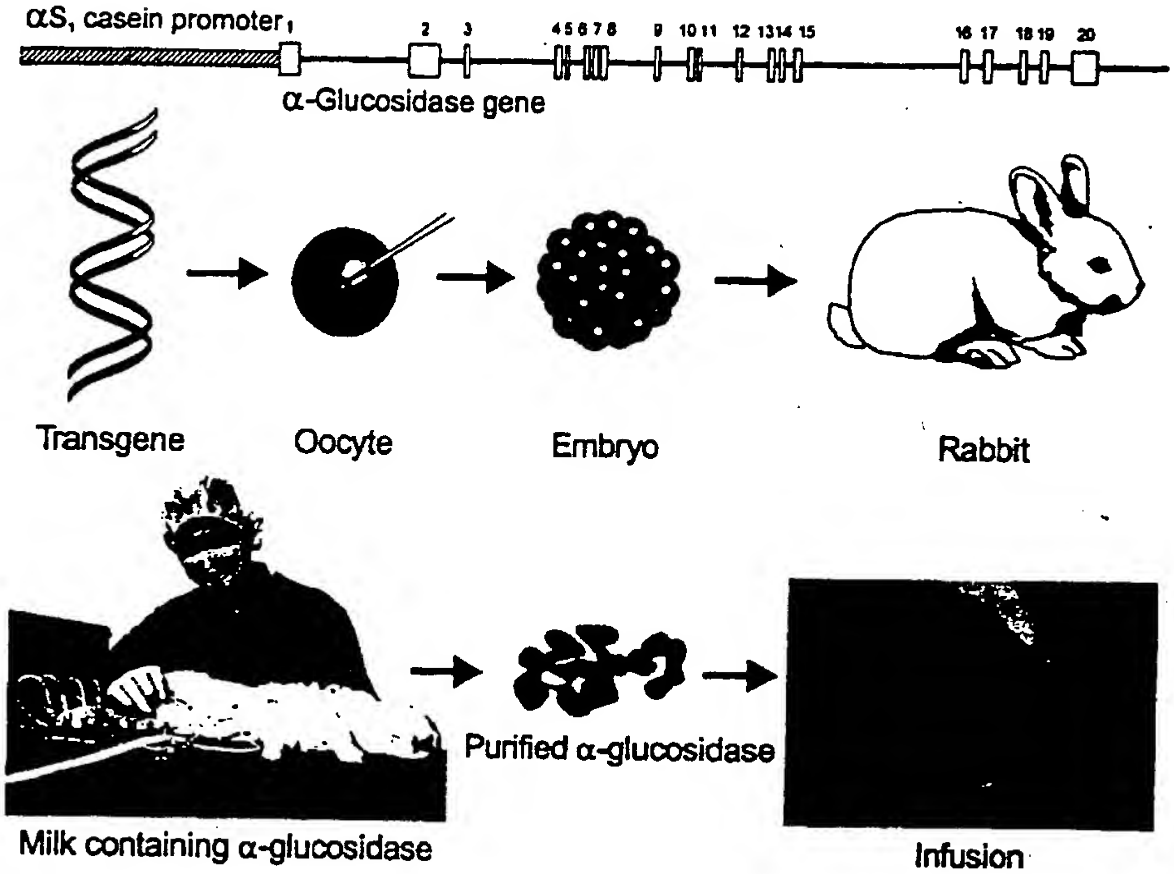
Technical challenges and financing

It took another 8 years before the first clinical trial of enzyme therapy in Pompe disease started. The time was spent on the development of technology to produce therapeutic grade recombinant human AGLU on a large scale. Therapy for Gaucher disease started with the heroic action of Genzyme Corporation (Boston, USA) to produce tailor-made glucocerebrosidase from tons of human placentas. The investment paid off for all parties. Patients with non-neurological forms of Gaucher disease (type 1) obtained an effective medicine [12]. Scientists were pleased because their ideas were realised. The company had shown its strength by bringing a new drug on the market and obtained returns.

The Orphan Drug Legislation, lending certain attractive rights to companies marketing medicines for rare diseases, has played an important role in this development. The past 5 years have shown concerted action of scientists, patients, industry and investors to be a golden formula for developing enzyme therapy for lysosomal storage diseases. Belief in enzyme therapy has returned. More importantly, the first reports confirming efficacy have appeared and clinical trials are ongoing for at least four of the lysosomal storage diseases [1, 10, 20, 28, 31]. In all instances, the protocols are directed to receptor-mediated tissue targeting, and recombinant DNA technology is applied for controlled large-scale enzyme production. The investment climate is excellent. The market seems profitable enough to have several companies competing for the same product. Both TKT (Boston, USA) and Genzyme Corporation have launched enzyme therapy for Fabry disease. Four companies are presently engaged in the development f enzyme therapy for Pompe disease. Genzyme/Pharming/Synpac as conglomerate and Novazyme (Oklahoma City, USA) as newcomer on the lysosomal disease market. As of August 2001, Genzyme and Novazyme have merged.

Back in 1991 there was no company strong and experienced enough to take up the challenge of producing recombinant acid AGLU for the treatment of Pompe disease, but research continued. Two Chinese hamster ovary (CHO) cell lines expressing high levels of recombinant enzyme were produced in university centres using the same AGLU cDNA but different vector systems [11, 36]. Using our CHO cell line, we produced a sufficient amount of enzyme to deliver proof of the principle of enzyme therapy in a mouse model of Pompe disease [7]. Simultaneously, therapeutic effect was shown in Japanese quails with the disease using the other CHO cell line [22]. Meanwhile, a completely different technology emerged which involved the production of medicines in the milk of transgenic animals [18]. Genomic DNA constructs are typically used in this production process in contrast to cDNA constructs employed in CHO cells (Fig. 2). The acid *AGLU* gene is linked to the promoter region of the bovine α_{S1}-casein gene that promotes high level expression in epithelial cells of the mammary gland. The construct is introduced in the animals' genome by injection into the pronucleus of fertilised oocytes. Embryos are implanted in foster mothers and a line of transgenic animals is obtained by germline transmission. The enzyme is harvested from the milk. Both production systems require downstream processing. The recombinant human acid AGLU from either milk or CHO cell media needs to undergo several rounds of purification before it can be administered intravenously to patients. The end products from CHO cells and milk are very similar in molecular mass (110 kD), and kinetic properties [7, 11, 36]. The carbohydrate compositi n may vary slightly depending on the enzyme source.

Fig. 2. Recombinant human AGLU from rabbit milk. The human acid *AGLU* gene is fused to 6.3 kb of the bovine α_{S1}-casein promoter and as such injected in the pronucleus of fertilised rabbit oocytes to generate transgenic founders. A transgenic line is obtained by breeding. Females produce recombinant human enzyme in the mammary gland during lactation and secrete the product in their milk. The enzyme is extracted from the milk in several purification steps and administered intravenously to the patients



Recombinant acid AGLU from CHO cells and mouse milk, both produced in our own laboratory, are equally efficiently taken up by cultured fibroblasts of patients with Pompe disease via the M6P receptor. However, the same enzyme from rabbit milk is taken up less efficiently by cultured fibroblasts. Interestingly, the uptake of the two enzymes by the target tissues of mice is not consistently different (unpublished results). More extensive studies are needed to verify these initial findings.

Clinical studies

Recombinant human acid AGLU from both sources were finally tested in the clinic (Table 1). Dr Chen of Duke University, North Carolina, USA reported at the SHS symposium in Fulda the results of his study with CHO enzyme. We have summarised below the design and outcome of our study in which the enzyme from rabbit milk was used [31].

Study design and outcome

The aim of the study was to test safety and efficacy of recombinant human AGLU from rabbit milk in patients with the severe infantile form of Pompe disease. For inclusion, patients had to have the combination of generalised muscle weakness, cardiomegaly, acid AGLU deficiency and glycogen storage in skeletal muscle. Patients older than 10 months and those who were dependent on artificial ventilation were excluded. Four patients were included; two with an advanced stage of disease (7 and 8 months old), and two younger patients who were in significantly better condition at inclusion (2.5 and 3 months old). The two older patients were practically immobile at the time of inclusion. They required supplemental oxygen and had signs of cardiac instability. One of them became respirator dependent directly after inclusion before the start of treatment. The other patient became ventilator dependent after 10 weeks of treatment during a bout of pneumonia. One of the younger patients had signs of cardiac decompensation and respiratory distress at birth and was fed by nasogastric tube. The fourth patient was diagnosed at birth when he showed cardiomegaly on a chest X-ray film that was taken for disease unrelated indication. Both younger patients manifested axial hypotonia, head lag and slipping through.

The recombinant human AGLU from rabbit milk was administered intravenously in a weekly dose of 15–20 mg/kg (at start of treatment) to 40 mg/kg (at present) and is generally well tolerated. Transient reactions are seen sometimes during infusion, such as fever, malaise, erythematous rash, sweating, flushing and tachycardia. All are manageable without medication. After 12 weeks of treatment with the low dose, the acid AGLU activity in muscle had increased from 1%–2% of normal (before treatment) to 12%–28%, the levels typically measured in late-onset Pompe disease. During the 12 following weeks with high dose, the activity increased to normal levels. After 36 weeks of treatment, we observed improvement in muscle morphology in the younger patient who was in the best condition at the start of treatment. Muscle tissue sections stained less intense for glycogen (PAS staining) and muscle fibres appeared less damaged. Similarly clear

changes were at that time not yet evident in muscle biopsies of the other three patients.

Cardiac changes were monitored by ultrasound. The left ventricular mass index of all patients, exceeding approximately three times normal at inclusion, decreased after start of treatment (up to 25% of baseline for one of the patients). Signs of cardiac instability disappeared in all cases.

All patients gained strength over the first 36 weeks of treatment. They learned to play with toys. The two younger patients perform better than the two older ones. One of the younger patients learned to lift her legs from the surface and touch her feet whilst playing. At present she can sit independently. The other, with the best condition at start of treatment, has learned to sit and crawl at 9.5 months of age. At 12 months he could creep and stand with support of one arm and he could walk at 16 months. Importantly, all patients have well passed the age of 1 year which is more than the average life expectancy of patients with infantile Pompe disease. All four patients receive continuous treatment to evaluate the long-term effect of enzyme replacement therapy on motor and mental development and overall quality of life.

Results of two trials

Comparing the studies with recombinant human AGLU from rabbit milk and CHO cells, there are differences and similarities in outcome. One patient in each study responds very well. The patient treated with rabbit milk enzyme had a characteristic cardiomegaly at birth. The patient treated with CHO enzyme had a normal baseline cardiac evaluation with a left ventricular mass at two standard deviations above the norm (close to the P98) when treatment was started at 3 months after birth. Obviously, we are confronted with clinical diversity preventing in part the comparison of data. Further, dose level and infusion frequencies were different in the two trials. A second patient in the study with rabbit milk enzyme responds well in that she has acquired the ability to roll over and sit and has remained ventilator independent over the first 72 weeks. The patient is homozygote for the deltaT525 mutation and does not produce endogenous acid AGLU. Her progress demonstrates that a cross-reactive immunological material (CRIM) negative status is not a priori inhibitory for successful treatment. In contrast, the two CRIM negative patients in the trial with CHO enzyme were said to respond initially well to the treatment, but their condition declined when high antibody titres developed against the recombinant enzyme. The other patients in both trials are CRIM positive to some extent. The difference in antibody response is multi-interpretable. First of all, the enzyme preparations used are probably not identical with respect to precise molecular structure, notably the carbohydrate composition. Second, the two preparations may contain various types and degrees of impurities and are formulated differently. This may affect their immunogenicity. Moreover, the dosing regimen was different at the two test sites. All these factors, separate or together, may explain why CRIM negative patients respond differently to the two enzyme preparations.

Prospects and challenges

The positive effects of enzyme therapy for Pompe disease are too strong to deny. For the benefit of patients, enzyme therapy ought to be brought to the market. It requires a Phase III trial to deliver final proof of therapeutic effect. The current pilot studies with rabbit milk and CHO enzyme indicate that quick and c nvincing results can be obtained by extension of studies in patients with the infantile form of Pompe disease; but, at the same time, it seems inevitable that not all included patients will respond equally well. The problem can in part be managed by careful definition of inclusion and exclusion criteria and proper dosing. A second challenge is to prove efficacy of enzyme therapy in late onset Pompe disease. The milder affected patients live longer and are continuously confronted with loss of quality of life. It is essential for them to implement therapy at the earliest possible moment in order to prevent irreversible damage of muscle function. In theory, it is easier to correct the enzyme deficiency in late onset than in early onset disease because the residual AGLU activity is significantly higher in the former than in the latter condition. On the other hand, it has to be awaited whether adult muscle tissue is equally accessible for the enzyme and equally reparable as growing muscle of infants.

As it stands, the prospects of enzyme therapy for Pompe disease are good, but hurdles still need to be overcome. In April 2000, Genzyme-Pharming LLC announced the discontinued development of enzyme replacement therapy with recombinant human AGLU from rabbit milk. The companies stated that they believed production in CHO cells to be quicker. A new study with enzyme from CHO cells was started in May 2001. All together, that is more than 35 years after the first trials were undertaken. Patients, investigators and companies are eagerly awaiting the outcome.

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PREFACE

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PATENT & TRADEMARK OFFICE

PATENT APPLICATION
Docket No.: 2984.1000-004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant:	Yuan-Tsong Chen		
Application No.:	09/902,461	Group:	1654
Filed:	July 10, 2001	Examiner:	M. Meller
Confirmation No.:	6796		
For:	TREATMENT OF GLYCOGEN STORAGE DISEASE TYPE II		

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BRIEF ON APPEAL

Mail Stop Appeal Brief-Patents
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Sir:

This Brief on Appeal is submitted pursuant to the Notice of Appeal received in the U.S. Patent and Trademark Office on October 6, 2003, and in support of the appeal from the final rejections set forth in the Office Action mailed on June 3, 2003. The fee for filing a brief in support of an appeal is enclosed.

I. REAL PARTIES IN INTEREST

The real parties in interest are: Duke University, Erwin Road, Durham, North Carolina, Assignee of the entire right, title and interest in the subject application by virtue of an Assignment recorded on January 8, 2002 at Reel 012446, Frames 0057-0059; Synpac (North Carolina), Inc., 200 Meredith Drive, Durham, North Carolina, Licensee of the subject in the

application; and Genzyme Corporation, One Kendall Square, Cambridge, Massachusetts, Sub-Licensee of the subject application.

II. RELATED APPEALS AND INTERFERENCES

Appellant, the undersigned Attorney, Assignee, Licensee and Sub Licensee are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-9 and 11-23 have been finally rejected, and a copy of these claims appears in Appendix A of this Brief. Claims 1, 8, 9, 21 and 22 were amended in the Amendment filed on September 16, 2002, and Claims 1, 11-15, 21 and 22 were amended in the Amendment filed on March 3, 2003. Claims 2-7 and 16-20 appear as originally filed. Claim 10 was canceled in the Amendment filed on September 16, 2002.

IV. STATUS OF AMENDMENTS

A Remarks After Final Rejection was submitted by facsimile on October 16, 2003, and was entered as indicated in the Advisory Action mailed from the U.S. PTO on November 18, 2003.

V. SUMMARY OF INVENTION

Appellant's invention includes a method of treating glycogen storage disease type II (GSD-II), also known as Pompe's Disease, in a human individual. In the method, a therapeutically effective amount of acid α-glucosidase (GAA) is administered to the individual periodically at an administration interval. The administration interval can be varied, depending on the severity of the symptoms and the needs of the individual. The GAA is human acid α-glucosidase (hGAA) produced in Chinese hamster ovary (CHO) cell culture. One advantage of production of GAA in CHO cell culture is that it is believed to yield hGAA having a glycosylation pattern which allows significant and efficient uptake of the hGAA in the desired tissues (heart and muscle) and resultant cleavage of glycogen, to treat GSD-II. See the

specification, for example, at p. 2, lines 22-25; p. 3, line 25, through p. 5, line 22; p. 5, line 23, through p. 6, line 13; p. 9, lines 4-23 and 24-28.

Appellant's invention includes a method of treating cardiomyopathy associated with GSD-II in a human individual by administering to the individual a therapeutically effective amount of hGAA periodically at an administration interval, wherein the hGAA was produced in CHO culture (Claim 21). Appellant's invention also includes a pharmaceutical composition comprising hGAA which was produced in CHO cell culture, in a container, the container having a label with instructions for administration of the composition for treatment of GSD-II. See the specification, for example, at the above pages as well as at p. 4, lines 23-26, and p. 10, lines 10-14.

VI. ISSUES

The following issues remain on appeal:

1) Whether Claims 1-9 and 11-23 are vague and indefinite under 35 U.S.C. §112, second paragraph, in view of the use of the word, "periodically;"

2) Whether Claims 1-4, 9, 21 and 23 are anticipated by Fuller *et al.* under 35 U.S.C. 102 (b);

3) Whether Claims 1-7, 11-18, 21 and 23 are anticipated by Fuller *et al.* under 35 U.S.C. 102 (b), or in the alternative, obvious over, Fuller *et al.* under 35 U.S.C. 103 (a);

4) Whether Claims 1-9 and 11-23 are obvious over Fuller *et al.* under 35 U.S.C. 103 (a); and

5) Whether Claims 1-9 and 11-23 are obvious over Bijvoet *et al.* in view of Fuller *et al.* under 35 U.S.C. 103 (a).

VII. GROUPING OF CLAIMS

With regard to issue 1, the claims do not stand or fall together: Claims 1-9, 11-21 and 23 are grouped together, and Claim 22 stands separately. With regard to the remaining issues (2 through 5), the claims do not stand or fall together. Based on the rejections set forth in the Office Action, made final, and for the purposes of this Appeal, Claims 1-9, 11-20 and 23 are grouped

together; Claim 21 stands separately; and Claim 22 stands separately. Discussion of the reasons why the claims of each of these group are separately patentable are included in the arguments presented below.

VIII. ARGUMENT

Issue 1

The Examiner rejected Claims 1-9 and 11-23 under 35 U.S.C. §112, second paragraph, stating "periodically" was vague and indefinite. This rejection is improper.

Words not specifically defined in the specification should be read as they would be by one of ordinary skill in the art. (M.P.E.P. Section 2111.01). Furthermore, a dictionary is an appropriate objective resource that can serve as a reliable source of information on established meaning that could be attributed to a claim term by one of skill in the art (see, e.g., Intellectual Property Development Inc. v. UA-Columbia Cablevision of Westchester Inc., 68 USPQ2d 1385 (CAFC 2003)). The Merriam-Webster Dictionary indicates that the term, "periodically," means "at regular intervals of time." A copy of the Dictionary entry was attached to the Remarks After Final Rejection (filed in the US PTO on October 16, 2003) as Exhibit A.

Further, the Specification provides several examples of what is intended by the word "periodically." See, e.g., p. 3, line 1, where it says "periodically (e.g., monthly, bimonthly, weekly, biweekly). See also p. 9, line 21 where it says "periodically (as distinguished from a one-time dose)". This language supports the ordinary definition of the word, periodically, as it is set forth in the dictionary. Thus, one of ordinary skill in the art would understand that "periodically" refers to administration at intervals of time. This is further supported by the additional claim language regarding administration "at an administration interval." Furthermore, one of ordinary skill in the art, given the Specification, would be able to determine the appropriate interval for a particular patient (see, e.g., p. 9, lines 24-29).

Appellant's Attorney also notes that the word, "periodically" has been used in the claims of granted U.S. patents relating to therapeutic application or administration of drugs or medications, without any further definition of the term in the specifications of such patents. See, for example, US Patents 4,735,802; 4,749,708; 4,761,417; 4,833,132; 5,292,754; 6,133,317; and 6,464,994. Copies of the claims of these Patents are attached in Appendix B.

For these reasons, the word "periodically" in Claims 1-9 and 11-23 does not make these claims vague and indefinite under 35 U.S.C. 112, second paragraph. Lastly, the word, "periodically," does not appear in Claim 22; therefore, this rejection is inapplicable to Claim 22.

Issue 2

The Examiner rejected Claims 1-4, 9, 21 and 23 under 35 U.S.C. §102(b) as being anticipated by Fuller *et al.*, Reference AV2. This rejection is also legally improper.

In order for a reference to anticipate a claim, each and every element at set forth in the claim must be found, either expressly or inherently described, in the reference (see, e.g., M.P.E.P. §2131). The following elements of the claims are neither expressly or inherently described by Fuller *et al.*: 1) administration of GAA *to a human individual*; 2) administration *periodically* and *at an administration interval*; 3) *treatment* of GSD-II in a human individual.

Fuller *et al.* describe preparation of recombinant GAA in CHO cell culture. Fuller *et al.* further indicate that the recombinant GAA was taken up in two types of cells from a patient having Pompe's disease: in cultured human skin fibroblasts after exposure to the enzyme for 12 hours, as well as in cultured human muscle cells after exposure to the enzyme for 24 hours. Lysosomal glycogen in the muscle cells was cleared, following addition of recombinant GAA to the culture medium of the cells. Fuller *et al.* do not describe administration to a human individual: they do not describe administration of GAA to anything other than cells in culture. Furthermore, Fuller *et al.* do not describe administration of GAA periodically, nor do they describe administration at an administration interval. In addition, Fuller *et al.* do not describe "treatment" of disease in an individual, as that term is described in the current Specification (see, e.g., p. 4, line 20 *et seq.*) and would be understood by one of ordinary skill in the art. Neither uptake of enzyme by cultured human fibroblasts *in vitro*, nor uptake of enzyme by cultured human skeletal muscle cells and subsequent processing of lysosomal glycogen in the muscle cells *in vitro*, both occurring in the short term (e.g., 12 to 24 hours), provides an indication whether administration of the GAA to a human patient, periodically, at an administration interval, will treat the disease. It is known in the art that *in vitro* cell culture conditions differ significantly from *in vivo* conditions: for example, when enzyme is administered *in vivo* by intravenous infusion, the muscle cells don't come into direct contact with enzyme as they do in cell culture.

Furthermore, the endothelial barrier, as well as the interstitial connective tissue, must be passed *in vivo*. See, for example, Reuser *et al.* (*Eur. J. Pediatr.* 161:S106-S111 (2002); a copy of which was attached to the Remarks After Final Rejection as Exhibit C) .

Claim 21 differs from Claims 1-4, 9 and 23 in that it pertains specifically to treatment of cardiomyopathy associated with GSD-II. Fuller *et al.* does not describe the impact of administration of enzyme to cardiac cells. Neither uptake of enzyme by cultured human fibroblasts, nor uptake of enzyme by cultured human skeletal muscle cells and subsequent processing of lysosomal glycogen in the muscle cells, as taught by Fuller *et al.*, provides any indication about the effects of the enzyme on cardiac cells. Furthermore, even if Fuller *et al.* did provide *in vitro* data regarding cardiac cells, such data would not indicate whether human heart cells *in vivo* would be able to uptake enzyme upon administration of the GAA to a patient and treat cardiomyopathy, because it is known in the art that *in vitro* cell culture conditions differ significantly from *in vivo* conditions, as stated above.

Despite the Examiner's statements, Fuller *et al.* do not teach each and every aspect of the claimed invention. Therefore, the claims are not anticipated by the teachings of Fuller *et al.*

Issue 3

The Examiner rejected Claims 1-7, 11-18, 21 and 23 under 35 U.S.C. §102(b) as anticipated by, or in the alternative, under 35 U.S.C. §103(a) as obvious over, Fuller *et al.*

The discussion regarding anticipation by Fuller *et al.* above similarly applies to the anticipation rejection of these claims. The rejection is incorrect.

With regard to the obviousness rejection, Fuller *et al.* state that they believe that the precursor GAA "will be a useful candidate for replacement therapy in GSD II patients."

In order for a reference to provide a legally sufficient basis for an obviousness rejection under Section 103, the reference must contain teachings to a person of ordinary skill that provide both (1) a motivation or suggestion to try and (2) a basis for a reasonable expectation of success. (see, e.g., M.P.E.P. §2143). Fuller, *et al.*, provide neither.

In regard to a suggestion to try, Fuller, *et al.* was published in 1995. Yet, despite the lack of any available treatment for this terrible disease, there are no published reports that *in vivo* research was attempted until Dr. Chen did so, about 5-6 years later. Those skilled in the art

looking for a treatment for Pompe's Disease clearly did not see the teachings of Fuller, *et al.* as a suggestion to treat humans having this disease with periodic administrations of a hGAA produced in CHO cells, as was done by Dr. Chen. Furthermore, as discussed above, Fuller *et al.* provide no motivation to administer enzyme periodically, at an administration interval, as they do not teach administration of enzyme more than once, nor do they suggest that periodic administration should be performed, or that administration at an interval should be used.

Assuming *arguendo* that one of ordinary skill in the art was motivated to try to treat Pompe's disease by this teaching of Fuller *et al.*, the current invention would nevertheless not have been obvious, because one of ordinary skill in the art would not have had a reasonable expectation that treatment would be successful.

The terrible effects of Pompe's disease, failure of other alternative treatments, and Dr. Chen's successful treatment are described by the Muscular Dystrophy Association (MDA) in the publication QUEST (Volume 10, Number 2, March/April 2003). A copy of this publication was left as a courtesy with the Examiner during the interview on September 17. A copy was also attached to the Remarks After Final Rejection as Exhibit B and is attached to this Brief as Appendix C.

Pompe's disease is a rare disease which is extremely devastating for the afflicted individuals; infants with the disease are not expected to live beyond one year of age. The disease has been known for about seventy years, since the early 1930's, and the enzyme deficiency has been known for about forty years, since the 1960's. And yet, many attempts at treatment of the disease by administration of replacement enzyme have failed. See, for example, Van der Ploeg *et al.* (*J. Clin. Invest* 87:513-518 (1991), cited in IDS as reference AW3, which lists several references that indicate that attempts at enzyme replacement therapy have failed); Williams *et al.* (Birth Defects: Original Article series Volume XVI, no. 1, pp. 415-423 (1980), cited in IDS as reference AW, which states that a preliminary trial to treat a terminally ill patient with Pompe disease was not clinically successful); and de Barsy *et al.* (Birth Defects: Original Article Series, Vol IX. No. 2, pp. 184-190(1973), cited in IDS as reference AU2, which states that no conspicuous morphologic or clinical improvements were noted after an attempt to treat a patient with enzyme; that no morphologic or biochemical evidence of replacement therapy had been

obtained to date; and that it appeared that the enzyme was not being transported to the relevant places in the body).

Dr. Chen demonstrated successful treatment of this genetic disease by administration of the enzyme to a human individual. As described in detail in the Example in the application, periodic administration of GAA produced in Chinese hamster ovary cells to three separate patients, resulted in significant amelioration of symptoms associated with the disease, as well as delay in onset of more severe symptoms. For example, significant improvements in cardiac parameters were noted in all patients; pulmonary function and skeletal muscle functions improved and remained normal in one patient; neurologic and developmental characteristics were either improved, or remained normal. The successful reversal of certain symptoms in all patients, as well as the normal muscle functions, neurologic and developmental characteristics of the third patient, were highly significant because it was previously unknown whether human symptoms could be alleviated or whether normal development could be achieved by administration of GAA. Without treatment, these children were expected to die; as reported in the attached MDA article, most infants with the disease aren't expected to live to one year of age. Dr. Chen's invention, on the other hand, is a successful treatment of an otherwise fatal genetic disease affecting heart and muscle tissues. The unexpected nature of this success is further emphasized in the Declaration under 37 C.F.R. §1.132 of Dr. Chen (the "Appellant"), previously submitted.

Furthermore, as noted above, the teachings of Fuller *et al.* provide no information on whether heart cells would be able to uptake and use the enzyme upon administration *in vivo.* With regard to the method of Claim 21, one of ordinary skill in the art would not have known or expected that administration of the enzyme to a human individual would result in successful treatment of cardiomyopathy associated with this genetic disease.

In view of the long-felt need for a treatment and the failure of others to treat Pompe's disease successfully, including attempts to treat the disease by enzyme replacement, one skilled in the art would not have had a reasonable expectation of success from the teachings of Fuller, *et al.* This, coupled with Dr. Chen's unexpected success, make the claimed invention non-obvious over the teachings of Fuller *et al.* under 35 U.S.C. 103 (a).

Issue 4

The Examiner also rejected Claims 1-9 and 11-23 under 35 U.S.C. §103(a) as being obvious over Fuller *et al.*, stating specifically that it would have been obvious to use an immunosuppressant with the enzyme to treat the disease, so that the body would be less likely to reject the enzyme and more readily use it to treat disease. The only claims that recite an immunosuppressant are Claims 19 and 20. Thus, it would seem that the rejection should only apply to these claims.

The M.P.E.P. indicates that "in considering the disclosure of a reference, it is proper to take into account not only specific teachings of the reference, but also the inferences which one skilled in the art would reasonably be expected to draw therefrom" (M.P.E.P. 2144.01, quoting *In re Preda*, 401 F.2d 825, 826, 159 USPQ 342, 344 (CCPA 1968)). While Fuller *et al.* describe preparation of recombinant GAA in CHO cell culture, and indicate that the recombinant GAA was taken up in two types of cells from a patient having Pompe's disease (cultured human skin fibroblasts and cultured human muscle cells), Fuller *et al.* do not teach or suggest that rejection of enzyme *in vivo* would be a concern; in fact, they do not describe use of the enzyme *in vivo* at all. Because there are no immune cells in the cell culture used by Fuller *et al.*, one of ordinary skill in the art would not expect or infer any results regarding an immune response to the enzyme. Thus, given the teachings of Fuller *et al.*, one of ordinary skill in the art would not be expected to draw an inference that rejection of enzyme *in vivo* would occur at all, as there is no teaching regarding any activity of the enzyme *in vivo* and no teaching regarding any immunoreactivity of the enzyme. In view of these considerations, the invention of Claims 19 and 20 would not have been obvious over the teachings of Fuller *et al.*

Issue 5

The Examiner rejected Claims 1-9 and 11-23 under 35 U.S.C. 103(a) as being unpatentable over Bijvoet *et al.*, Reference AR2, in view of Fuller *et al.*, stating that it would have been obvious to use the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*

Bijvoet *et al.* describe production of transgenic recombinant hGAA in mouse milk; administration of a single dose of GAA to GSD-II knockout mice, and a resultant increase of enzyme activity in homogenized heart and skeletal muscle samples after two days; and the uptake

of the enzyme by cultured human fibroblasts. Bijvoet *et al.* do not describe administration of GAA to a human individual, as is required by the claims; in addition, Bijvoet *et al.* do not teach or suggest administration of GAA periodically, at an administration interval. Furthermore, they do not describe "treatment" of disease as it is described in the Specification and would be understood by one of ordinary skill in the art.

One of ordinary skill in the art, given the teachings of Bijvoet *et al.*, would not have been motivated to look to the teachings of Fuller *et al.* regarding enzyme produced in CHO cell culture; in fact, Bijvoet *et al.* teach away from use of enzyme produced in CHO cells: they indicate that high production costs associated with use of enzyme produced in CHO cells are a significant concern and discuss experiments designed to provide proof of principle for obtaining enzyme by other means (Bijvoet *et al.*, p. 1820, "Discussion").

Even assuming *arguendo* that the teachings of Bijvoet *et al.* were combined with the teachings of Fuller *et al.*, one of ordinary skill in the art would not have obtained the present invention. One of ordinary skill in the art, using the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*, would have been motivated only to administer a single dose of the enzyme, and not to administer enzyme periodically at an administration interval.

Furthermore, given the teachings of Bijvoet *et al.* in combination with the teachings of Fuller *et al.,* one would not have had a reasonable expectation of successfully treating a human individual. As discussed above, there was a long-felt need for a treatment and significant failures by others to achieve treatment of Pompe's Disease by any means, including administration of enzyme. One of ordinary skill in the art would not have known, given the teachings of Bijvoet *et al.* regarding uptake of enzyme by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme, whether periodic administration, at an administration interval, to a human with Fuller *et al.*'s GAA, would successfully "treat" the patient, as treatment is described in the Specification and would be understood by one of ordinary skill in the art.

Furthermore, Even assuming *arguendo* that the teachings of Bijvoet *et al.* were combined with the teachings of Fuller *et al.*, one of ordinary skill in the art would not have obtained the present invention as set forth in Claim 21. One of ordinary skill in the art, using the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*,would not have known whether administration *in*

vivo would successfully treat cardiomyopathy, because one would not have had a reasonable expectation of success. The teachings of Bijvoet *et al.* describe uptake of enzyme by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme; if these teachings were combined with the teachings of Fuller *et al.* regarding the enzyme, the combination does not provide a reasonable expectation that administration of the enzyme periodically to a patient at an administration interval will, in fact, treat cardiomyopathy. Bijvoet *et al.* describe increased activity in homogenized mouse heart and muscle tissue; these experiments do not indicate whether the enzyme has located to the relevant cells (e.g., myocytes), that will allow it to treat disease (e.g., by decreasing glycogen and/or decreasing symptoms). In fact, as supported by Dr. Chen's Declaration, one of ordinary skill in the art would expect that intravenous administration of enzyme would result in the presence of the enzyme in the blood stream and endothelium of blood vessels, rather than in the desired target cells. Furthermore, even if the enzyme located to the desired cells, Bijvoet *et al.* do not demonstrate a decrease of glycogen or other correction of symptoms.

As discussed above, there was a long-felt need for a treatment of Pompe's Disease (GSD-II) and significant failures by others in the art to achieve treatment. One of ordinary skill in the art would not have known, given the teachings of Bijvoet *et al.* regarding uptake of enzyme (produced in milk) by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme, whether periodic administration, at an administration interval, to a human individual, of the GAA as taught by Fuller *et al.,* would in fact result in successful treatment of cardiomyopathy as set forth in Claim 21.

With regard to Claim 22, Fuller *et al.* in combination with Bijvoet *et al.* do not teach or suggest a pharmaceutical composition comprising human acid α- glucosidase, wherein the human acid α- glucosidase was produced in chinese hamster ovary cell culture, in a container, the container having a label containing instructions for administration of the composition for treatment of GSD-II. As discussed in detail above, the combination of Fuller *et al.*, and Bijvoet *et al.* does not describe treatment of GSD- II, as the term "treatment" is understood by one of ordinary skill in the art. Therefore, it would not have been obvious to one of ordinary skill in the art to prepare a pharmaceutical composition in a container having a label with instructions for administration of the composition for treatment of the disease, as one would not have known

whether it was even possible to treat the disease: one would not have been able to prepare the label.

In view of these considerations, the claimed invention would not have been legally obvious under Section 103 over the teachings of Bijvoet *et al.* in combination with Fuller *et al.*

CONCLUSION

For the reasons presented above, Appellant's Attorney respectfully requests the Board of Patent Appeals and Interferences to reverse all of the rejections in the Office Action of June 3, 2003.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.
David E. Brook, R.N. 22592

By for Elizabeth W. Mata
Elizabeth W. Mata
Registration No. 38,236
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Date: 1/6/03

APPENDIX

1. (Twice Amended) A method of treating glycogen storage disease type II in a human individual having glycogen storage disease type II, comprising administering to the individual a therapeutically effective amount of human acid glucosidase periodically at an administration interval, wherein the human acid α- glucosidase was produced in chinese hamster ovary cell cultures.

2. The method of Claim 1, wherein the glycogen storage disease type II is infantile glycogen storage disease type II.

3. The method of Claim 1, wherein the glycogen storage disease type II is juvenile glycogen storage disease type II.

4. The method of Claim 1, wherein the glycogen storage disease type II is adult-onset glycogen storage disease type II.

5. The method of Claim 1, wherein the therapeutically effective amount of human acid α-glucosidase is less than about 15 mg of acid α-glucosidase per kilogram of body weight of the individual.

6. The method of Claim 5, wherein the therapeutically effective amount of human acid α-glucosidase is about 1-10 mg of acid α-glucosidase per kilogram of body weight of the individual.

7. The method of Claim 5, wherein the therapeutically effective amount of human acid α-glucosidase is about 5 mg of acid α-glucosidase per kilogram of body weight of the individual.

8. (Amended) The method of Claim 1, wherein the human acid glucosidase is recombinant human acid glucosidase that has been produced in chinese hamster ovary cell cultures.

9. (Amended) The method of Claim 1, wherein the human acid glucosidase is a precursor of recombinant human acid glucosidase that has been produced in chinese hamster ovary cell cultures.

11. (Amended) The method of Claim 1, wherein the administration interval is monthly.

12. (Amended) The method of Claim 1, wherein the administration interval is bimonthly.

13. (Amended) The method of Claim 1, wherein the administration interval is weekly.

14. (Amended) The method of Claim 1, wherein the administration interval is twice weekly.

15. (Amended) The method of Claim 1, wherein the administration interval is daily.

16. The method of Claim 1, wherein the human acid α-glucosidase is administered intravenously.

17. The method of Claim 1, wherein the human acid α-glucosidase is administered intramuscularly.

18. The method of Claim 1, wherein the human acid α-glucosidase is administered intrathecally or intraventricularly.

19. The method of Claim 1, wherein the human acid α-glucosidase is administered in conjunction with an immunosuppressant.

20. The method of Claim 19, wherein the immunosuppressant is administered prior to any administration of human acid α-glucosidase to the individual.

21. (Twice Amended) A method of treating cardiomyopathy associated with glycogen storage disease type II in a human individual having glycogen storage disease type II, comprising administering to the individual a therapeutically effective amount of human acid glucosidase periodically at an administration interval, wherein the human acid glucosidase was produced in chinese hamster ovary cell culture.

22. (Twice Amended) A pharmaceutical composition comprising human acid glucosidase, wherein the human acid glucosidase was produced in chinese hamster ovary cell culture, in a container, the container having a label containing instructions for administration of the composition for treatment of glycogen storage disease type II.

23. The method of Claim 1, wherein the administration interval is varied over time.

EXHIBIT A

Main Entry: pe·ri·od·i·cal·ly
Pronunciation: "pir-E-'ä-di-k(&-)lE
Function: adverb
Date: 1646
1 : at regular intervals of time
2 : from time to time : FREQUENTLY

1. Pronunciation Key

EXHIBIT B

is kept in place undisturbed until completely dry. Optionally, it can be removed aseptically by suitable means such as washing and drying the skin. Conveniently the mask can be applied and set before bedtime in the evening and during the night, allowed to fall off or slough off as when completely dry. The second application of the mask typically can be undertaken in a short period, e.g. with the next few hours. To a considerable extent, the effectiveness of the therapy depends on the number of successive applications. Typically, a beneficial result can be observed within several days, usually within about 3 to 5 days, in a regimen where the treatment is given each day or preferably each night, as described. The papules first lose their edematous, erythematous appearance, they then shrink and finally disappear. For difficult, deepseated inflammatory lesions, the progressive healing starts as the reduction of firmness, then shrinkage of lesions, and the eventual disappearance of the lesions, with absence of or minimal scarring.

In another aspect, the invention concerns a dermatological composition adapted for topical treatment of dermatoses of the kind described, comprising a sterile homogeneous therapeutic paste made by mixing a water component and dried calcium sulfate in fine powder form that essentially is an odorless, tasteless hemihydrate. In one preferred embodiment, the composition is packaged with the water component and the dried powder component in separate sealed containers. The paste is of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing. The water component in one preferred embodiment optionally contains a water soluble thickener such as sodium carboxymethyl cellulose in an amount sufficient to cause the water component to gel and prevent the water component from flowing. The water component suitably may contain a self sterilizing agent which may be conventional and preferably is trichlocarban, oxychlorosene sodium or triclosan constituted in dilute solution e.g. 0.1–0.2%. Suitable topical anti-infectives are described in The Nurses Guide to Drug Therapy, Chapter 88, Prentice-Hall, Inc., 1984.

The paste in one preferred embodiment includes a per se effective amount of a compatible anodyne such as zinc oxide. As constituted if necessary with buffering, the paste is essentially at neutral pH.

A preferred compositon for treating dermatoses is a smooth workable paste made by mixing sterile water with heat-sterilized calcium sulfate hemihydrate, in the weight ratio of 4 parts to about one part; optionally with thickener, buffer, anti-infective agent and/or anodyne.

Having thus described my invention, the embodiments in which an exclusive property or privilege is claimed are defined as follows:

1. A method of treating dermatoses that are characterized by lesion sites, exudate, and chronic inflammation of the sebaceous glands and follicles of the skin, including the steps of:

(a) applying a sterile homogeneous settable therapeutic paste composition consisting essentially of a mixture of water and dried calcium sulfate, to the skin area comprising the lesion sites in a co-extensive overlying soft mask or layer,

(b) allowing the mask to set until hard and to remain in place on the skin for a time sufficient to become dry and achieve absorption and adsorption of exudate,

(c) allowing the mask to slough off or optionally removing the thus sorbed exudate by aseptic removal of the mask from the skin;

and, if necessary repeating the regimen of steps a, b and c periodically until remission of the lesions is achieved.

2. The method of treating acne according to claim 1 where the regimen of steps a, b and c is carried out once daily for several days until remission of the lesions is achieved.

3. The method of treating acne according to claim 1 where the regimen of steps a, b and c is carried out once each night for several nights until remission of the lesions is achieved.

4. A dermatological composition adapted for topical treatment of dermatoses that are characterized by lesion sites, exudate and chronic inflammation of the sebaceous glands and follicles of the skin, consisting essentially of a sterile homogeneous therapeutic paste made by mixing a water component containing a self-sterilizing agent in an amount sufficient to maintain sterility of the composition, and dried calcium sulfate in fine powder form that essentially is an odorless tasteless hemihydrate, the paste being of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing.

5. A composition according to claim 4 where the self-sterilizing agent is selected from trichlorcarban, oxychlorosene sodium and triclosan.

6. A composition according to claim 4 where the paste includes a per se effective amount of a compatible anodyne agent.

7. A composition according to claim 4 where the paste is essentially at neutral pH.

8. A dermatological composition adapted for topical treatment of dermatoses that are characterized by lesion sites, exudate and chronic inflammation of the sebaceous glands and follicles of the skin, consisting essentially of a sterile homogeneous therapeutic paste made by mixing a water component and dried calcium sulfate in fine powder form that essentially is an odorless tasteless hemihydrate, the paste being of a non-flowing consistency that can be worked or formed into a layer covering a selected area of the skin and being settable to a hard mass on standing, the water component containing water soluble carboxymethyl cellulose in an amount sufficient to cause the water component to gel and prevent the water component from flowing.

9. A composition according to claim 8 where the paste includes a self-sterilizing agent.

* * * * *

These dogs were instrumented with an electromagnetic flowmeter placed around the ascending aorta to measure cardiac output, with catheters in the left ventricle and the aorta for recording of their respective pressure. All these parameters together with left ventricular dP/dt and lead aVF of the electrocardiogram were recorded during the experiments continuously at a speed of 1 mm/second and at 15 minute intervals at a speed of 200 mm/second.

Coreximine was given orally at doses of 0.25, 0.5 and 5 mg/kg. Each dose was studied in 6 dogs. At a dose of 5 mg/kg the total amount of the compound delivered approximates the amount delivered by infusion at 0.2 mg/kg/min for 30 minutes (6 mg/kg).

The dose of 0.25 mg/kg was only mildly effective. At the dose of 0.5 mg/kg, the left ventricular dP/dt increased by about 30% from 2618 ± 221 to 3556 ± 371 mm Hg/sec ($P<0.05$). Cardiac output increased significantly from 2.4 ± 0.1 to 2.9 ± 0.1 1/min ($P<0.01$) and left ventricular and diastolic pressure fell from 6.7±0.8 to 5.8±0.6. At the same time mean arterial pressure increased by 20 mm of Hg while heart rate increased from 73 to 94 beats per minute. Therefore, oral coreximine, even at a very low dose, is a positive inotropic agent improving left ventricular performance and is well absorbed orally. It should be noted, however, that there was some increase in heart rate and blood pressure.

At the higher dose of 5 mg/kg, which corresponds to 0.17 mg/kg/min, the dP/dt increased from 2559±76 to 4630±378 mm Hg/sec ($P<0.005$), an increase of about 80%. The systolic arterial pressure increased from 125 to 168 mm Hg while the diastolic arterial pressure increased from 78 to 106 mm Hg. Heart rate increased from 83 to 133. Therefore, while heart rate and blood pressure increased, the dP/dt increased markedly. The cardiac output increased from 2.2±0.1 to 2.6±0.1 ($P<0.005$) and total peripheral resistance increased by 22%. Left ventricular and-diastolic pressure fell from 6.4±0.6 to 4.4±0.7 ($P<0.05$).

Thus, coreximine increased strikingly contractility, decreased the preload and increased cardiac output thus augmenting performance while increasing arterial pressure and heart rate.

The data, which shows an increase in heart rate and increase in arterial pressure when coreximine is administered orally in contrast to a decrease in these two effects when the drug is administered intravenously suggests that the increase in the heart rate and arterial pressure is due to the bitterness of the berberine derivatives or some other outside influence. In the above experiments the natural bitterness of coreximine was not masked by conventional means known to those skilled in the art.

Next, coreximine was given orally to dogs that were pretreated with propanolol at a dose of 1 mg/kg. Propanolol was given to verify whether coreximine has a beta adrenergic agonist activity, specifically to verify whether the increased in contractility measured by dP/dt max and dP/dt at 40 mm Hg of developed pressure would disappear. It was found that the increase in contractility does not disappear after this beta blockade and therefore the increase in contractility is not due to beta angonist effects either of the drug itself or other external influences. The increases in blood pressure also were not nullified; however, increases in blood pressure are alpha and not beta mediated.

In another experiment, 13-allylberberine bromide was given orally to conscious dogs at a dose of 5 and of 50 mg/kg. Each dose was given to 5 dogs. The compound is effective orally when given 50 mg/kg but not when given 5 mg/kg. When given 50 mg/kg it increased dP/dt significantly from 2561 to 3391 mm Hg/sec, showing a marked positive inotropic property. Also, cardiac output increased from 2.3 to 2.7 l/min and left ventricular end-diastolic pressure fell from 5.S to 4.5 mm Hg showing an improvement in ventricular performance and a reduction in preload. Heart rate increased from 69 to 81 and mean arterial pressure from 90 to 116 mm Hg. The increase in heart rate and arterial pressure again suggests the influence of the bitterness of the drug.

Moreover, when 2 doses of 13-allylberberine bromide 50 mg/kg were given orally with an interval of 30 minutes between them dP/dt increased from 2500 to 4600 mm Hg, an increase of 84% showing that higher or multiple dose may be more effective.

Similarly other pharmaceutically acceptable salts of 13-allylberberine produce equivalent effects.

13-Methylberberine citrate given 50 mg/kg orally in 2 dogs increased left ventricular dP/dt by an average of 27% suggesting that this compound was absorbed when given orally. When it was given intravenously 0.2 mg/kg/min for 30 minutes its effect was similar suggesting around a 10:1 intravenous to oral ratio of effectiveness.

Berberine tartrate (5 mg/kg) given orally to 2 dogs increased left ventricular dP/dt by 17%.

None of the above mentioned compounds caused arrhythmias. The compounds of the instant invention have either an antiarrhythmic effect or no obserable effect on arrhythmias at the dosage at which the compounds are administered in the above experiments.

I claim:

1. A therapeutic method of increasing the contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of coreximine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

2. The therapeutic method of claim 1 wherein the compound is administered periodically.

3. The therapeutic method of claim 1 wherein the composition is administered by intravenous infusion.

4. A therapeutic method of increasing the contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering orally to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of coreximine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

5. The therapeutic method of claim 4 wherein the compound is administered periodically.

6. The method of claims 1, 2, 3, 4 or 5 wherein the compound is administered in a dosage in the range of about 0.2 to 5 mg/kg.

* * * * *

TABLE XIV-continued

Effect of Berberine on Ejection Fraction							
	Ejection fraction (quadruplicate reading)					Mean	Average
5 min	30 min	.43	.47	.45	.42	.44	
infusion	45 min	.35	.39	.37	.39	.38	
Berberine	5 min	.45	.46	.50	.43	.46	0.40 ± 2
0.7 mg/kg/min	15 min	.37	.39	.37	.35	.37	(p < 0.05)
	30 min	.41	.41	.38	.38	.40	
	45 min	.36	.39	.42	.35	.38	

TABLE XV

Effect of Berberine (0.2 mg/kg/min) on Ejection Fraction (as done by MUGR) in Conscious Dogs with Acute Left Ventricular Failure (LVF).

	Before LVF	LVF	During Berberine Infusion	30 min after Berberine Infusion
Dog #				
1	45	24	38	33
2	56	34	51	41
3	53	35	49	44
4	58	20	31	30
5	55	21	35	30
6	45	28	39	33
Average ± SE	55 ± 2	27 ± 2	40 ± 3	35 ± 2
	p < 0.001		p < 0.001	
			p < 0.001	

TABLE XVI

Effects of Berberine and Ouabain on Hemodynamics in Dogs with Acute Heart Failure

	Control		Oubain		Berberine	
	Before	ΔChange	Before	ΔChange	Before	ΔChange
Heart rate (beats/min)	136 ± 4	0 ± 3	124 ± 5	−3 ± 1*	126 ± 12	−8 ± 2*
Systolic AP (mmHg)	124 ± 9	−1 ± 4	115 ± 6	1 ± 3	118 ± 7	−4 ± 3
Diastolic AP (mmHg)	97 ± 9	−1 ± 4	84 ± 7	3 ± 2	90 ± 7	−9 ± 3*
Mean AP (mmHg)	111 ± 9	−3 ± 4	98 ± 7	0 ± 2	102 ± 7	−6 ± 2*
Pulse pressure (mmHg)	26 ± 1	0 ± 1	31 ± 3	−1 ± 2	28 ± 2	5 ± 3
LV dP/dt (mmHg/sec)	1628 ± 132	−72 ± 64	1700 ± 80	178 ± 61*	1572 ± 80	440 ± 91 ‖ **
LV EDP (mmHg)	14.1 ± 0.5	0.4 ± 0.5	13.0 ± 1.8	−2.0 ± 1.1	13.3 ± 0.8	−5.0 ± 0.8 ‖ **
Cardiac output (liter/min)	1.77 ± 0.28	−0.22 ± 0.08*	1.84 ± 0.16	−0.07 ± 0.18	1.76 ± 0.15	0.17 ± 0.06 **
Stroke volume (ml/beat)	12.9 ± 1.8	−1.4 ± 0.5	14.8 ± 1.1	−0.2 ± 1.4	14.6 ± 1.9	2.7 ± 1.1 *
TPR (dyn.sec.cm^{-5})	5943 ± 1124	601 ± 466	4495 ± 551	10 ± 491	4915 ± 559	−852 ± 156 **

AP = Aortic pressure; LV dP/dt = Maximal rate of rise of left ventricular pressure; LV EDP = left ventricular end diastolic pressure; TPR = Total peripheral vascular resistance; * = $p < 0.05$ and ** = $p < 0.005$ for paired t-test between values before and after intervention; = $p < 0.05$ and = $p < 0.01$ for unpaired t-test between control group and treated groups; ‖ = $p < 0.05$ for unpaired t-test between berberine HCl and oubain treated groups.

TABLE XVII

Effect of Berberine, Oubain and the Combination of Both Peak LV dP/dt (numbers are percent increase compared to before treatment).

Drug Intervention	Time after Treatment 5 min	10 min	15 min	20 min	25 min	30 min
Berberine alone	30.2 ± 4.1	36.2 ± 3.5	39.2 ± 3.3	42.6 ± 4.9	38.8 ± 5.4	40.5 ± 6.7
Ouabain alone	17.1 ± 2.1	24.0 ± 2.8	33.8 ± 3.8	36.1 ± 4.3	44.0 ± 6.3	49.0 ± 7.3
Ouabain and Berberine	xxx	xxx	xxx	xxxx	xxxx	xxxx
	87.1 ± 10.0	82.0 ± 12.8	87.5 ± 13.3	95.4 ± 16.0	98.2 ± 16.5	97.6 ± 14.7
	ooo	ooo	ooo	oooo	oooo	oooo
Ouabain and Berberine vs. Berberine alone	= p < 0.005 ooo					
	p < 0.000 oooo					
Ouabain and Berberine vs. Ouabain alone	= p < 0.005 xxx					
	P < 0.001 xxxx					

I claim:

1. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of berberrubine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing administration.

2. The therapeutic method of claim 1 wherein the compound is administered periodically.

3. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof in an amount effective to cause a positive inotropic effect, a composition which comprises a biologically acceptable carrier and a compound selected from the group consisting of tetrahydropalmatine and its pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing administration.

4. The therapeutic method of claim 3 wherein the compound is administered periodically.

5. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof a composition which comprises a pharmaceutically acceptable carrier and a cardiac glycoside and in conjunction therewith, in an amount effective to cause a positive inotropic effect, a composition which comprises a pharmaceutically acceptable carrier and a compound selected from the group consisting of berberrubine, tetrahydropalmatine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect.

6. The therapeutic method of claim 5 wherein the compound is administered periodically.

7. The therapeutic method of claim 5 wherein the compound is berberrubine or its pharmaceutically acceptable salts.

8. The therapeutic method of claim 5 wherein the compound is tetrahydropalmatine or its pharmaceutically acceptable salts.

9. The therapeutic method of claim 5, 6, 7 or 8 wherein the cardiac glycoside is selected from the group consisting of ouabain, digoxin, digitoxin and delanoside.

10. A therapeutic method for increasing contractility of the mammalian heart as shown by a positive inotropic effect which comprises administering to a mammal in need thereof a composition which comprises a pharmaceutically acceptable carrier and a cardiac glycoside and in conjunction therewith, in an amount effective to cause a positive inotropic effect, a composition which comprises a pharmaceutically acceptable carrier and a compound selected from the group consisting of berberine and the pharmaceutically acceptable salts thereof, causing a positive inotropic effect and discontinuing the administration of the composition comprising the cardiac glycoside and the compositiion comprising the compound.

11. The therapeutic method of claim 10 wherein the compound is administered periodically.

12. The therapeutic method of claim 10 or 11 wherein the cardiac glycoside is selected from the group consisting of ouabain, digoxin, digitoxin and delanoside.

13. The method of claims 1, 2, 3 or 4 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

14. The method of claims 1, 2, 3 or 4 wherein the compound is administered in a dosage in the range of about 0.02 to 0.7 mg/kg.

15. The method of claim 5 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

16. The method of claim 5 wherein the compound is administered in a dosage in the range of about 0.02 to about 0.7 mg/kg.

17. The method of claim 10 wherein the compound is administered in a dosage in the range of about 0.001 to about 50 mg/kg.

18. The method of claim 10 wherein the compound is administered in a dosage in the range of about 0.02 to 0.7 mg/kg.

* * * * *

treated eye) of 3 points at 2, 4 and 6 hours after application.

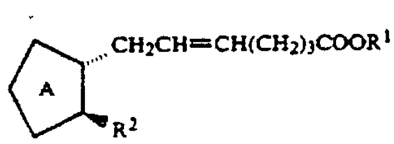
TABLE 11

	difference (mmHg)						
days	0	14	28	42	56	70	84
Sodium salt of 6	2.20	2.02	2.24	2.03	2.01	2.09	2.14
timolol maleate	2.73	2.54	1.10	1.26	1.20	1.26	1.22

It is known that the intraocular pressure-reducing effect of timolol maleate, which is most broadly used in clinical field, is lowered in association with the lapse of the application term. The results in Table 11 shows that the intraocular pressure-reducing effect of the sodium salt of the compound 6 is not lowered even if it is used in a long duration, which is an advantage of the compound of the present invention over timolol maleate.

What is claimed is:

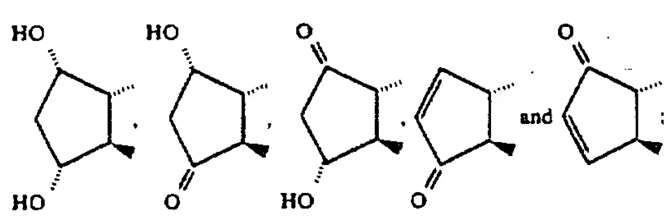
1. A method for treating hypertension or glaucoma in the eye comprising contacting the surface of the eye with a therapeutic amount of a 15-deoxyprostaglandin derivative of the formula (I):



(I)

in which

is a 5 membered ring which is selected from a group consisting of

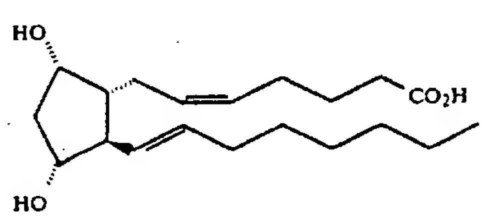


R^1 is hydrogen or lower alkyl;

R^2 is C_6-C_{12} alkyl, C_6-C_{12} alkenyl or C_6-C_{12} alkadienyl or a pharmaceutically acceptable salt thereof.

2. The method as claimed in claim 1, wherein the compound as indicated in claim 1 is periodically contacted with the surface of the eye.

3. The method as claimed in claim 1, wherein (5Z,13E,9S,11R)-9,11-dihydroxy-5,13-prostadiehoic acid of the formula (I):



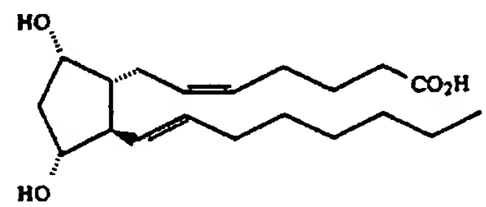
or a pharmaceutically acceptable salt or a lower salt alkyl ester is periodically contacted with the surface of the eye at a dose of 1 μg/eye/day to 1000 μg/eye/day.

4. A kit for delivery of a topical solution for treatment of hypertension or glaucoma in the eye which comprises:

(a) container having a solution including a compound of the formula (I), and

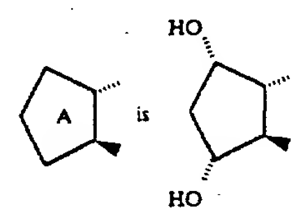
(b) means for topical delivery of said solution to the eye in a controlled dosage.

5. The kit as claimed in claim 4, wherein the compound is (5Z,13E,9S,11R)-9,11-dihydroxy-5,13-prostadienoic acid of the formula (I):



or a pharmaceutically acceptable salt or a lower alkyl ester.

6. The method as claimed in claim 1, wherein



7. The method as claimed in claim 1, wherein R^2 is C_6-C_{12} alkenyl.

8. The method as claimed in claim 7, wherein R^2 is C_8-C_{10} alkenyl.

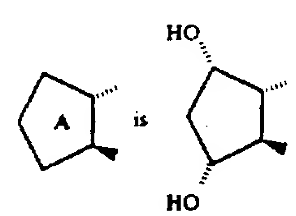
9. The method as claimed in claim 8, wherein R^2 is a straight-chain C_8-C_{10} alkenyl.

10. The method as claimed in claim 6, wherein R^2 is C_6-C_{12} alkenyl.

11. The method as claimed in claim 10, wherein R^2 is C_8-C_{10} alkenyl.

12. The method as claimed in claim 11, wherein R^2 is a straight-chain C_8-C_{10} alkenyl.

13. The kit as claimed in claim 4, wherein



14. The kit as claimed in claim 4, wherein R^2 is C_6-C_{12} alkenyl.

15. The kit as claimed in claim 14, wherein R^2 is C_8-C_{10} alkenyl.

16. The kit as claimed in claim 15, wherein R^2 is a straight-chain C_8-C_{10} alkenyl.

17. The kit as claimed in claim 13, wherein R^2 is a C_6-C_{12} alkenyl.

18. The kit as claimed in claim 17, wherein R^2 is C_8-C_{10} alkenyl.

19. The kit as claimed in claim 18, wherein R^2 is a straight-chain C_8-C_{10} alkenyl.

* * * * *

TABLE XI

VITAMIN B-6 (PYRIDOXINE) CONTENT OF SELECTED BAKED GOODS

Breads	Mg/100 G
Bagels: Plain, Onion, Poppy Seed, Sesame	.051
Bagels, Date Bran	.000
Bagels, Egg	.084
Bagels, Cinnamon Raisin	.000
Biscuits, Commercially Baked Plain or Buttermilk	.047
Cornbread	.113
Cracked Wheat Bread	.304
French and Vienna Bread	.043
Italian Bread	.048
Mixed Grains 7 Bread	.333
Oat Bran Bread	.000
Pumpernickel	.126
Rye Bread	.075
Wheat Bran Bread	.064
Whole Wheat Commercial Bread	.179

Source: U.S. Department of Agriculture
Agriculture Handbook 8–18 Rev. 1992

TABLE XII

Dry Dog Food
Test Run Data

E325 Processing Conditions:	Run #1	Comment
Product	Dry Dog Food	
Feeder Speed (RPM)	11 rpm (420 lbs/hr)	
Feeder Speed (Hertz)	34.7	
Cond. Cyl. Temp (° F.)	206	
Cond. Cyl. Water (% gauge)	10	
Extruder Speed (RPM)	421	
Extruder Current (Amps)	24	
Extruder Water (% gauge)	0	
Extruder Steam Injection	0	
#2 Head Temp. (CW or ST)	CW	
#3 Head Temp. (CW or ST)	CW	
#4 Head Temp. (CW or ST)	CW	
#5 Head Temp. (° F.)	CW 79 deg F.	
#6 Head Temp. (° F.)	CW 129 deg F.	
Die Pressure (PSI)	350	
Knife Speed (Hertz)	55.9	
Dryer Temperature (° F.)	223	
Dryer Retention (minutes)	17.9	
Formula:	See Dry Feed formula 2.2% Oxalic acid solution added at 0.7 lbs/min	2.7# OA to 100# water
Comments:	The oxalic acid solution was made up in hot water (160° F.) and pumped into the conditioning cylinder	
Screw Configuration:		
#1 Screw	Single Flight Tapered Inlet	Straight Rib Head
#1 Steamlock	Spacer	
#2 Screw	Single Flight Uncut	Spiral Rib Head
#2 Steamlock	Spacer	
#3 Screw	Single Flight Uncut	Spiral Rib Head
#3 Steamlock	Spacer	
#4 Screw	Single to Double	Straight Rib Head

TABLE XII-continued

Dry Dog Food
Test Run Data

E325 Processing Conditions:	Run #1	Comment
	Flight Uncut	
#4 Steamlock	Small	
#5 Screw	2 Flight cut flight	Straight Rib Head
#5 Steamlock	Large	
#6 Screw	2 Flight cut flight cone	Spiral Rib Cone Head
Die Configuration:		
Spacer	1" thick	
Backup Die	No	
Dieplate	1 1/4" central insert die	#825440-3
Insert	1/4" round hole	#101-509

Thus, it will be appreciated that as a result of the present invention, a highly effective oxalic acid or oxalate composition and method is provided by which the principal object, among others, is completely fulfilled. It is contemplated, and will be apparent to those skilled in the art from the preceding description that modifications and/or changes may be made in the prescribed embodiments without departure from the present invention. Accordingly, it is expressly intended that the foregoing description is illustrative of preferred embodiments only, and not limiting with respect to the true spirit and scope of the present invention.

What is claimed is:

1. A chemopreventive composition for treating at least one of tumors, brain tumors, cancers, and growths in warm blooded animals sensitive to treatment comprising a composition having an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

2. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid in a free acid form.

3. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

4. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is dried parsley.

5. The composition as recited in claim 1 further comprising a pharmaceutically acceptable carrier or diluent.

6. The composition as recited in claim 1 wherein said carrier or diluent is distilled water.

7. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is a nutritional supplement containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

8. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is selected from the group of natural foods, processed foods, molds, plants, and vegetables containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

9. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid

and oxalate is selected from the group of beverages, liquids, and juices containing an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

10. The composition as recited in claim 1 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is selected from the group of additives containing at least one therapeutically effective form of at least one of oxalic acid and oxalate.

11. The composition as recited in claim 5, wherein said at least one carrier and diluent is a gel cap.

12. The composition as recited in claim 5, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

13. The composition as recited in claim 1, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

14. An anti-tumor agent to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising a mixture of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate with at least one of a pharmaceutically acceptable carrier and diluent.

15. The agent as recited in claim 14 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

16. The agent as recited in claim 14 wherein said pharmaceutically acceptable carrier or diluent is selected from the group of distilled water, heated water, pharmaceutically acceptable liquids, nutritional supplements, natural foods and processed foods.

17. The anti-tumor agent as recited in claim 14, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

18. The agent as recited in 14, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

19. A therapeutic composition in cream or ointment form for topical administration of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate, a solvent, and a base.

20. The therapeutic composition as recited in claim 19 wherein said solvent is at least one of distilled water, acetone, propylene glycol, and polysorbate, and said base is at least one of a cream, ointment, gel, lotion, spray, stick, and powder.

21. The therapeutic composition as recited in claim 19 wherein said solvent is at least one of distilled water, acetone, propylene glycol, and polysorbate and said base is at least one of a hydrophilic petrolatum, cream, ointment, gel, lotion, spray, stick, and powder.

22. The composition as recited in claim 19, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

23. In a pet food containing at least one of protein, carbohydrates, oils, vitamins, and minerals, to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment, the improvement comprising the addition of a therapeutically effective quantity of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

24. The pet food as recited in claim 23, wherein the effective quantity is about 1 mg to 3 g per pet.

25. In a method of manufacturing a dry process dog food to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment, the improvement comprising the steps of mixing a slurry of conventional dog food ingredients together with a dilute solution of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate in heated water to form at least one of an oxalic acid and oxalate containing slurry, forming the slurry into pellets and drying the pellets.

26. The method as recited in claim 25 wherein said oxalic acid is oxalic acid dihydrate and each pellet contains approximately 1 mg of oxalic acid so that one pound of dry dog food contains about 1 g of oxalic acid.

27. The method as recited in claim 25, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

28. An oral rinse or wash for treating at least one of tumors, cancers, and growths in warm blooded animals sensitive to treatment in the mouth area comprising a dilute solution of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said solution is adapted to be administered periodically in less than a lethal dosage.

29. The oral rinse or wash as recited in claim 28 comprising a dilute solution of oxalic acid dihydrate in distilled water.

30. The oral rinse as recited in claim 29, wherein said rinse contains a solution ratio of about 150 mg oxalic acid dihydrate in about 300 ml distilled water.

31. The oral rinse as recited in claim 28, wherein said rinse contains a solution ratio of about 150 mg oxalic acid dihydrate in about 300 ml distilled water.

32. A dietary supplement for treating a patient diagnosed with at least one of an active cancer, tumor, and growth sensitive to treatment comprising about 1 g to 6 g of at least one therapeutically effective form of at least one of oxalic acid and oxalate per day based on 70 kilograms of body weight and at least one of a pharmaceutically acceptable carrier and diluent.

33. The dietary supplement as recited in claim 32, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule,

lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

34. The dietary supplement as recited in claim 32, wherein said effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

35. A pharmaceutical composition to be administered orally to humans for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising a mixture of a non-toxic ingestible carrier and an effective amount of a therapeutically effective form of at least one of oxalic acid and oxalate.

36. The pharmaceutical composition as recited in claim 35, wherein said at least one carrier and diluent is at least one of a gel cap, distilled water, tablet, powder, food additive, drops, liquid, beverage, rinse, mouthwash, gargle, pill, capsule, lozenge, cough drop, transdermal patch, ointment, salve, cream, lotion, and gel, adapted for periodically administering a therapeutically effective dosage by at least one of topical, oral, nasal, parenteral, intravenous, and subcutaneous application.

37. The composition as recited in claim 35, wherein said effective amount is about 50 mg to 6 g.

38. The pharmaceutical composition as recited in claim 35 wherein said composition is provided in a form selected from the group of pills, powders, granules, tablets, microcapsules, gel capsules, nutritional supplements, processed foods, liquids, drops, beverages, additives, and solutions.

39. In a pet treat containing at least one of protein, carbohydrates, and flavorings, to be used in a method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment w, the improvement comprising the addition of microgram amounts of at least one therapeutically effective form of at least one of oxalic acid and oxalate, whereby the treats provide for the maintenance of good pet health.

40. The pet treat as recited in claim 39, wherein the microgram amount provides for about 1 mg to 3 g per pet per day.

41. A chemopreventive composition for treating at least one tumor in a warm blooded animal sensitive to treatment comprising a composition including an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

42. The chemopreventive composition as recited in claim 41, wherein the effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

43. A chemopreventive composition for treating at least one cancer in a warm blooded animal sensitive to treatment comprising a composition including an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage.

44. The chemopreventive composition as recited in claim 43, wherein the effective amount is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

45. In a gel cap, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

46. The gel cap as recited in claim 45, wherein the effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

47. In a pill, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

48. The pill as recited in claim 47, wherein the effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

49. In a drug delivery system, the improvement comprising a therapeutically effective dosage of oxalic acid less than a lethal dosage.

50. The drug delivery system as recited in claim 49, wherein the therapeutically effective dosage is at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

51. A method for treating at least one of tumors, growths, and cancers in warm blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate to said animal, wherein said dosage is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered periodically in less than a lethal dosage.

52. The method as recited in claim 51 wherein said composition is administered at least one of orally and sublingually.

53. The method as recited in claim 51 wherein said composition is administered by injection.

54. The method as recited in claim 51 wherein said composition is administered topically.

55. The method as recited in claim 51 wherein said composition is administered internally.

56. The method as recited in claim 51 wherein said composition is administered at least once a day at a dosage of at least one of 50 mg to 6 g for humans and 1 mg to 3 g for warm blooded animals other than humans.

57. The method as recited in claim 51 wherein said composition is administered at least once a day at a dosage of about 1 mg to 3 g for dogs and cats.

58. The method as recited in claim 51, wherein said composition is administered by injection.

59. The method as recited in claim 51, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

60. The method as recited in claim 51 further comprising the steps of reducing the intake of oxalic acid or oxalate blockers.

61. The method as recited in claim 60 wherein said blockers are selected from the group of citric acid, ascorbic acid, pyridoxine hydrochloride, calcium, alcohol, resins, clays, and combinations thereof.

62. The method as recited in claim 60 wherein said blockers are selected from the group of dairy products containing calcium, fruits, coconut, beverages containing alcohol, ascorbic acid or citric acid including adult beverages such as beer, wine, vodka, gin, and the like, fruit juice based beverages, soda pop or soft drinks containing ascorbic acid or citric acid, other sports drinks, beverages or refreshments containing calcium, ascorbic acid or citric acid, red meat or white meat of fowl including chicken, turkey, pheasant and the like containing pyridoxine hydrochloride, or other foods or beverages containing alcohol, citric acid, ascorbic acid, calcium or pyridoxine hydrochloride including breads or grains, and combinations thereof.

63. A method for treating at least one of cancers, tumors, and growths in warm blooded animals sensitive to treatment comprising the steps of adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

64. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid in a free acid form.

65. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is a nutritional supplement containing at least one form of at least one of oxalic acid and oxalate.

66. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is oxalic acid dihydrate.

67. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is processed foods containing at least one form of at least one of oxalic acid and oxalate.

68. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is at least one of plants and vegetables containing at least one form of at least one of oxalic acid and oxalate.

69. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is at least one of beverages, liquids, and juices containing at least one form of at least one of oxalic acid and oxalate.

70. The method as recited in claim 63 wherein said therapeutically effective form of at least one of oxalic acid and oxalate is additives containing at least one form of at least one of oxalic acid and oxalate.

71. The method as recited in claim 63, wherein said effective amount is a daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

72. The method as recited in claim 63, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

73. The diet as recited in claim 63 further comprising the steps of reducing the intake of oxalic acid or oxalate blockers.

74. The diet as recited in claim 73 wherein said blockers are selected from the group of citric acid, ascorbic acid, pyridoxine hydrochloride, calcium, alcohol, resins, clays, and combinations thereof.

75. The diet as recited in claim 73 wherein said blockers are selected from the group of dairy products containing calcium, fruits, coconut, beverages containing alcohol, ascorbic acid, or citric acid including adult beverages such as beer, wine, vodka, gin, and the like, fruit juice based beverages, soda pop or soft drinks containing ascorbic acid or citric acid, other sports drinks, beverages or refreshments containing calcium, ascorbic acid or citric acid, red meat or white meat of fowl including chicken, turkey, pheasant, and the like containing pyridoxine hydrochloride, or other foods or beverages containing alcohol, ascorbic acid, citric acid, calcium or pyridoxine hydrochloride including breads or grains, and combinations thereof.

76. A method of treating warm-blooded animals afflicted with tumor cells sensitive to an effective amount of at least one of an oxalic acid and oxalate compound comprising the steps of periodically administering to the animal an oncolytic amount of at least one therapeutically effective oxalic acid and oxalate compound.

77. The method as recited in claim 76, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

78. The method as recited in claim 76, wherein said compound is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

79. A method of treating brain tumors in warm blooded animals sensitive to treatment comprising the steps of ingesting or administering a therapeutically effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

80. The method as recited in claim 79 wherein the therapeutically effective form of at least one of oxalic acid and oxalate is dried chopped parsley.

81. The method as recited in claim 79, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

82. The method as recited in claim 79, wherein said effective form of at least one of oxalic acid and oxalate is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

83. A method for treating at least one of tumors, growths, and cancers in warm-blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of the composition of claim 1 and adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

84. The method as recited in claim 83, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid,

red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

85. The method as recited in claim 83, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

86. A method for treating cancer in warm blooded animals sensitive to treatment, comprising the steps of:

periodically administering a therapeutically effective dosage of at least one therapeutically effective form of at least one of oxalic acid and oxalate to said animal, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said solution is adapted to be administered periodically in less than a lethal dosage.

87. The method as recited in claim 86, wherein said composition is administered at least one of orally and sublingually.

88. The method as recited in claim 86, wherein said composition is administered by injection.

89. The method as recited in claim 86, wherein said composition is administered topically.

90. The method as recited in claim 86, wherein said composition is administered internally.

91. The method as recited in claim 86, wherein said composition is administered at least once a day at a dosage of at least one of 50 mg to 6 g for humans and 1 mg to 3 g for warm blooded animals other than humans.

92. The method as recited in claim 86, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

93. A method for treating at least one tumor in a warm blooded animal sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage to said animal.

94. The method as recited in claim 93, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

95. The method as recited in claim 93, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

96. A method for treating at least one cancer in a warm blooded animals sensitive to treatment comprising the steps of periodically administering a therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered to warm-blooded animals on a periodic basis in less than a lethal dosage to said animal.

97. The method as recited in claim 96, further comprising the steps of reducing the intake of oxalic acid or oxalate blockers selected from at least one of citric acid, ascorbic acid, (vitamin C), pyridoxine hydrochloride (vitamin B6), calcium, alcohol, resins, clays, foods containing calcium, beverages containing alcohol, citric acid, or ascorbic acid, red meat or white meat of fowl containing pyridoxine hydrochloride, and foods, nutritional supplements, or beverages containing oxalic acid or oxalate blockers.

98. The method as recited in claim 96, wherein said composition is administered at daily dosage of at least one of about 50 mg to 6 g for humans and about 1 mg to 3 g for warm blooded animals other than humans.

99. A treatment regimen for treating tumors, cancers, growths, and neoplasia in warm blooded animals sensitive to treatment comprising the steps of reducing or eliminating the ingestion or administration of oxalic acid or oxalate blockers, administering or ingesting high dosages of at least one of oxalic acid and oxalate to raise the blood or urine oxalic acid or oxalate level above normal, and, after cleansing the blood of tumor, cancer or abnormal cells, administering or ingesting a more moderate level of at least one of oxalic acid and oxalate to maintain a normal blood or urine oxalic acid or oxalate level.

100. The regimen as recited in claim 99 further comprising the steps of increasing the administration or ingestion of oxalic acid or oxalate enhancers.

101. A chemopreventive composition for treating at least one of tumors, brain tumors, cancers, and growths in humans sensitive to treatment comprising a composition having an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate and at least one of a carrier and diluent for said at least one of oxalic acid and oxalate, wherein said effective amount is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered on a periodic basis in less than a lethal dosage.

102. A method for treating at least one of tumors, growths, and cancers in humans sensitive to treatment comprising the steps of periodically administering at therapeutically effective dosage of at least one composition of a therapeutically effective form of at least one of oxalic acid and oxalate, wherein said dosage is less than a lethal dosage of oxalic acid and wherein said composition is adapted to be administered periodically in less than a lethal dosage.

103. The method as recited in claim 102 wherein said composition is administered at least one a day at a dosage of about 50 mg to 6 g.

104. A method for treating at least one of cancers, tumors, and growths in humans sensitive to treatment comprising the steps of adding to the regular diet a dietary supplement of an effective amount of at least one therapeutically effective form of at least one of oxalic acid and oxalate.

* * * * *

1. Simple ointment, oleaginous:

white wax 25 g

yellow wax 25 g

petrolatum 950 g

The proportion of wax can be varied to obtain a suitable consistency of the ointment.

2. Hydrophilic Petrolatum (this preparation will absorb large amount of water of aqueous solutions of active ingredient, form water-in-oil type emulsions):

Cholesterol 30 g

stearyl alcohol 30 g

white wax 80 g

white petrolatum 860 g

3. Lanolin, Hydrous or anhydrous wool fat.

4. Washable Hydrophilic Ointment (oil-in-water emulsion):

methylparben 0.025 g

propylparben 0.015 g

sodium lauryl sulfate 10 g

propylene glycol 120 g

stearyl alcohol 250 g

white petrolatum 250 g

purified water 370 g

5. Polyethylene Glycol Ointment:

polyethylene glycol 3350 350 g

polyethylene glycol 400 600 g

stearyl alcohol 50 g

6. Absorbent dusting powders

Magnesium stearate 100 g

7. Absorbent dusting powders:

Magnesium stearate 50 g

Boric Acid 50 g

8. Absorbent/adsorbent Delivery Particles:

Mixtures of porous and non-porous micro beads.

The formulations listed above are for illustrative purposes only. Moreover, the treating protocol used to treat or prevent diaper dermatitis can include one or more of the compositions of the present invention as well as one or more of the formulations set forth above. Of course, to the vehicles shown above may be added the enzyme inhibitors or inactivators and/or the additional therapeutically effective adjuvants as set forth herein to address the effects of diaper dermatitis.

The compositions or formulations of this invention are generally prepared by mixing an effective amount of an enzyme inhibiting system alone or in conjunction with an adjuvant system into a desired vehicle under conditions of time, temperature and pressure to facilitate the formation of a substantially homogeneous composition. Generally, the mixing time is between about 0.1 minutes and about 1 hour, preferably between about 0.2 minutes and about 40 minutes and particularly between about 1 minute and about 30 minutes. Generally, the temperature is between about 50° F. and about 300° F., preferably between about 60° F. and about 200° F., and particularly about room temperature. Generally, the pressure is between about 0.5 atmosphere and about 25 atmospheres, preferably between about 1 atmosphere and about 5 atmospheres, and particularly at about standard atmospheric pressure. The mixing can be carried out in a traditional open mixer, a mixing extruder, a blender or any other mixing apparatus well-known in the art. Of course, the mixing time, mixing temperature and mixing pressure can be adjusted to the particular equipment being used provided that the temperature does not exceed a decomposition temperature for the inhibitors or any other component of the composition.

One ointment formulation including an enzyme inactivating system of the present invention for direct application to skin follows:

8 g of cholestyramine (a weak anion resin)

50 g of Euserin a water based glycerin carrier.

200 mg of tetrahydrolipstatin

500 mg of trypsin-chymotrypsin inhibitor from Sigma Chemicals.

All references cited herein are incorporated by reference. While this invention has been described fully and completely, it should be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.

I claim:

1. A composition for treating enzyme induced tissue damage in an acidic environment comprising an effective amount of a treating system selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease, substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, wherein the composition neutralizes acid activity.

2. The composition of claim 1, wherein the enzyme inhibitor system comprises a lipase inhibitor.

3. The composition of claim 2, wherein the lipase inhibitor is selected from the group consisting of esterastin, lipstatin, valilactone, tetrahydrolipstatin, panclicin, ebelactone, ajoene and mixtures or combinations thereof.

4. The composition of claim 1, wherein the enzyme inhibitor system comprises a protease inhibitor.

5. The composition of claim 4, wherein the protease inhibitor comprises a trypsin-chymotrypsin inhibitor.

6. The composition of claim 1, wherein the enzyme inhibitor system comprises a lipase inhibitor and a protease inhibitor.

7. The composition of claim 6, wherein the lipase inhibitor is esterastin, lipstatin, valilactone, tetrahydrolipstatin, panclicin, ebelactone, ajoene or mixtures or combinations thereof and the protease inhibitor is a trypsin-chymotrypsin inhibitor.

8. The composition of claim 1, further comprises effective concentrations of therapeutic adjuvants, where the therapeutic adjuvants comprises anti-inflammatory agents, antimicrobial agents, antiboitic agents, hemostatic agents, anaesthetic agents, anti-fungal agents or mixtures or combinations thereof.

9. The composition of claim 1, further comprises a performance indicator to monitor the integrity of the enzyme inhibitor system.

10. The composition of claim 1, further comprising a carrier.

11. A method for treating enzyme damage to tissue in an acidic environment by:

administering to tissue of a subject an effective amount of an enzyme treating system, where the treating system is

selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, where the system neutralizes acid activity.

12. A method for treating enzyme damage to tissue in an acidic environment by:

periodically administering to a tissue of a subject an effective amount of an enzyme treating system, where the treating system is selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system, and combinations thereof, where the system neutralizes acid activity.

13. A method for preparing a composition for treating enzyme damage to tissue in an acidic environment by:

mixing an effective amount of an enzyme treating system selected from the group consisting of a sacrificial lipase substrate including hydrolyzable fat, sacrificial protease substrate including natural and synthetic preparations of amino acid residues, gastrins, and, gelatins, sacrificial lipase and protease substrate including bifunctional substrates having a fatty acid and amino acid moiety for decreasing enzyme activity, an enzyme inhibitor system and combinations thereof into a vehicle, where the system neutralizes acid activity.

* * * * *

invention to utilize a unit dosage form which will contain from about 250 mg. to about 800 mg. of 5-methyl-3-sulfanilamidoisoxazole or an equivalent amount of a salt thereof and from about 12.5 mg. to about 160 mg. of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or an equivalent amount of a salt thereof. The frequency with which any such unit dosage form will be administered to a warm-blooded animal will vary, depending upon the quantity of medicament present therein and the needs and requirements of the warm-blooded animal. Under ordinary circumstances, however, about a total of 60 mg./kg. of 5-methyl-3-sulfanilamidoisoxazole and about a total of 8 mg./kg. of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, in combination, can be administered daily in several doses.

As hereinabove discussed, detailed description is made with reference to unit dosages whether in intravenous or oral form, the frequency and dosage levels are best related with regard to the effectiveness in treating a fibrosarcoma (SAD_2) tumor in terms of component levels in the plasma of the warm-blooded animals being treated of the composition selected from the group consisting of 5-methyl-3-sulfanilamidoisoxazole, a salt of 5-methyl-3-sulfanilamidoisoxazole with a pharmaceutically acceptable base, 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, a salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine with a pharmaceutically acceptable acid and mixtures thereof. Generally, it is preferably desired to maintaini in the plasma of the warm-blooded animal a component level of the 5-methyl-3-sulfanilamidoisoxazole or an equivalent amount of the salt thereof of from about 80 to 160, preferably about 110 μg./cc. and/or a component level of the 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or an equivalent amount of the salt thereof of from about 5 to 15, preferably 10 μg./cc.

This invention relates to the invention described in copending applications (Ser. Nos. 655,079; 655,227; 655,145; and 655,080; now U.S. Pat. Nos. 4,632,920; 4,698,335; 4,632,919; and 4,661,478, respectively filed on even date herewith, the teachings of which are incorporated) by reference herein.

The foregoing, notwithstanding, it should be fully understood that the dosages set forth herein are exemplary only and they do not, to any extent, limit the scope or practice of the present invention. As indicated hereinbefore, the combination of this invention has unexpectedly been found to be particularly useful for its effects in the treatment of a fibrosarcoma (SAD_2) tumor in warm-blooded animals.

The invention will be understood better by reference to the following examples which are given for illustration purposes and are not meant to limit the invention.

EXAMPLES

CDF_1 mice (23 +2 gm.) were implanted with a fibrosarcoma (SAD_2) subcutaneously. One week later all mice received pentobarbital sodium anesthesia followed by either 0.1 cc. Injectable Composition (Trimethoprim 16 mg./cc., Sulfamethoxazole 80 mg./cc., Dosage: 320 mg./kgS, 64 mg./kgT) or an equal volume of saline. One hour later all mice received 400 rads (8MeV Photon) to the tumor.

TABLE

Two Week Survivals			
Saline	2/20	20%	$p < .05$

TABLE-continued

Two Week Survivals		
I.C.	5/11	46%

The two week survival time of recipients of the Injectable Composition was significantly greater than that of the control and suggest beneficial effects in neoplasia of such Injectable Composition.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

What is claimed:

1. A process for treating a warm-blooded animal having a fibrosarcoma (SAD_2) tumor which comprises administering to said animal
   (a) 400 rads of radiation,
   (b) from about 1 to about 30 parts selected from the group of 5-methyl-3-sulfanilamidoisoxazole and a alkali metal salt of 5-methyl-3-sulfanilamidosoxazole with a pharmaceutically acceptable base and,
   (c) from about 30 to about 1 part selected from the group of 2, 4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine and a salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine with a pharmaceutically acceptable acid,
   said compounds (b) and (c) being administered in an amount effective to lengthen the survival of said animal having a tumor.

2. The process as defined in claim 1 wherein the effective amount of the compounds administered comprise from about 250 mg to about 800 mg of 5-methyl-3-sulfanilamidoisoxazole or an alkali metal salt thereof with a pharmaceutically acceptable base and from about 12.5 mg. to about 160 mg. of 2,4-di-amino-5 (3,4,5-trimethoxy benzyl) pyrimidine or a salt thereof with a pharmaceutically acceptable acid.

3. The process of claim 1 wherein the compounds are administered intravenously.

4. The process of claim 1 wherein the compounds are administered orally.

5. The process of claim 1 wherein the pharmaceutically acceptable base with which the alkali metal salt of 5-methyl-3-sulfanilamidoisoxazole is formed is selected from the group consisting of sodium hydroxide and potassium hydroxide.

6. The process of claim 1 wherein the pharmaceutically acceptable acid with which the salt of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine is formed is a mineral acid or an organic acid.

7. The process of claim 6 wherein the mineral acid is selected from the group consisting of hydrochloric acid and sulfuric acid.

8. The process of claim 6 wherein the organic acid is selected from the group consisting of acetic acid, citric acid, lactic acid, maleic acid and salicyclic acid.

9. The process of claim 1 wherein the compounds are administered individually.

10. The process of claim 1 wherein the ratio of the amount administered of 5-methyl-3-sulfanilamidoisoxazole or an equivalent salt and the amount administered of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine is between about 5 and about 15 to 1.

11. The process of administering the compounds of claim 1 wherein up to about 160 mg/kg of 5-methyl-3-

sulfanilamidoisoxazole and up to about 8 mg/kg of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine are administered daily to said warm-blooded animal.

12. The process of claim 11 wherein the daily administration of the compounds is in several doses.

13. The process of claim 1 wherein the radiation is administered subsequent to the administration of the compounds.

14. A therapeutic method for treatment of a fibrosarcoma (SAD_2) tumor in a warm-blooded animal which comprises periodically administering to said warm-blooded animal 400 rads of radiation and a therapeutic composition which comprises a therapeutically effective amount of an antineoplastic/antimetastatic compound selected from the group consisting of 5-methyl-3-sulfanilamidoisoxazole or an alkali metal salt thereof with a pharmaceutically acceptable base sufficient to maintain a blood plasma concentration of said compound between about 80 and about 160 micrograms/cc.

15. The process as defined in claim 14 wherein a composition concentration is maintained at about 110 μg./cc.

16. A therapeutic method for treatment of a fibrosarcoma (SAD_2) tumor in a warm-blooded animal which comprises periodically administering to said warm-blooded animal 400 rads of radiation and a therapeutically effected amount of an antineoplastic/antimetastatic composition selected from the group consisting of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine or a salt thereof with a pharmaceutically acceptable acid sufficient to maintain a blood plasma concentration of said compound between about 5 and about 15 micrograms/cc.

17. The process as defined in claim 16 wherein a composition concentration is maintained at about 10 μg./cc.

* * * * *

QUEST Volume 10, Number 2, MARCH/APRIL 2003

Pompe's Disease

A Killer Yields to Modern Medicine

by *Dan Stimson*

You may never have heard of Pompe's disease. It affects just 5,000 to 10,000 people in the United States, making it exceedingly rare and of little interest to the general public. But what it lacks in notoriety, it makes up for in personal devastation to those who have it.

Robert Elmore nuzzles his son Grante ("Nikko"), who has lived twice as long as expected thanks to enzyme replacement therapy provided in a clinical trial.
Photos by Amy Snyder

Pompe's (also known as acid maltase deficiency) is caused by a genetic deficiency of an enzyme that breaks down glycogen (stored sugar) inside muscle cells. In its severest form, it strikes during infancy, weakening the heart and the voluntary muscles, including those that control breathing. The disease can also manifest during childhood or adulthood, causing significant muscle weakness and respiratory problems.

Children and adults with the disease usually have a shortened life span, and most infants with the disease aren't expected to live beyond 1 year of age.

But these grim prognoses could soon change, thanks to research led by Yuan-Tsong Chen, professor and chief of Medical Genetics in the Department of Pediatrics at Duke University in Durham, N.C., and director of the Institute of Biomedical Sciences, Academia Sinica, Taiwan. Through basic research supported by MDA and clinical trials supported by the biotech company Genzyme, Chen and his team at Duke have developed a way to supply the missing enzyme to people with Pompe's disease.

In two trials, one completed in 2000 and the other last year, 11 babies have received this experimental treatment — called enzyme replacement therapy — and some are now healthy, walking toddlers.

A Faulty Enzyme, Failing Muscles

With currently available treatment, "There's not much we can do for babies with Pompe's disease," Chen says.

Within weeks or months of birth, an infant with the disease can become too weak to suckle or breathe on its own. The muscular walls of the heart become enlarged, shrinking the heart's inner chambers and reducing its pumping capacity, a condition known as *hypertrophic cardiomyopathy*. Most babies with the disease die from cardiac and respiratory failure within three to four months of diagnosis, Chen says.

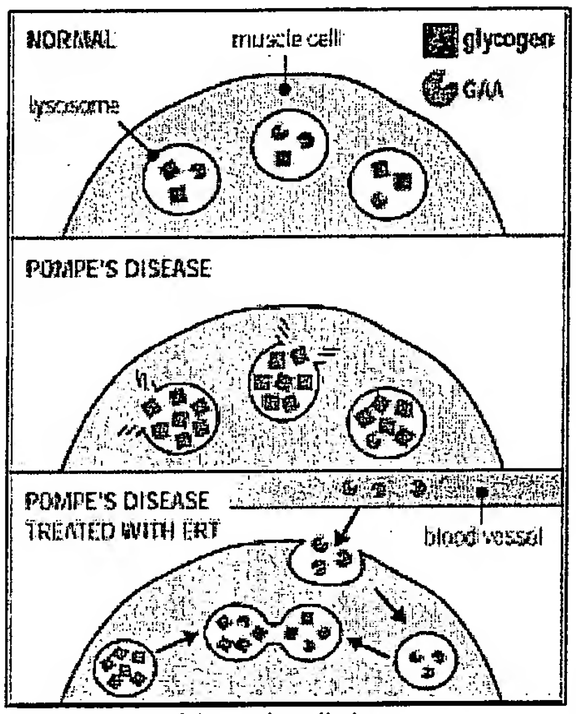
This picture of infant-onset Pompe's disease has changed little since it was first described by Dutch pathologist Joannes Pompe in the early 1930s. While studying at the University of Amsterdam, Pompe was asked to do an autopsy on a 7-month-old girl who had been admitted to the university hospital with difficulty breathing and had died three days later, apparently of pneumonia. Expecting to see her lungs filled with fluid, he was surprised to find that her heart had swollen to more than three times its normal size and the cells within it were filled with clumps of debris, which turned out to be glycogen.

It wasn't until the 1960s that other researchers discovered the underlying basis of Pompe's disease — a deficiency of the enzyme *acid alpha-glucosidase (GAA)*, also called *acid maltase*. The deficiency can now be detected by blood tests that probe for GAA activity or for mutations in the gene encoding GAA, located on chromosome 17. It's estimated that one in 85 to 100 people carries a mutation in a single copy of the gene; it takes mutations in both copies, one inherited from each parent, to cause the disease.

The severity of mutations in the GAA gene — that is, how much they alter the enzyme — determines, at least in part, the severity of the disease.

Mutations that destroy the protein cause infant-onset Pompe's disease, while mutations that leave some GAA intact tend to cause juvenile- and adult-onset forms of the disease.

The later-onset forms of Pompe's are primarily "muscle diseases," Chen says. Cardio-myopathy is mild in the juvenile form, and usually absent from the adult form. In children, the most common first symptom is delayed motor development; in adults, it's difficulty walking. For both late-onset forms, respiratory weakness can be severe and often requires mechanical ventilation, Chen says.



Working inside subcellular compartments called lysosomes, the enzyme acid alpha-glucosidase (GAA) breaks down glycogen (top). In Pompe's disease, a deficiency of GAA causes glycogen to accumulate and rupture lysosomes (middle). In enzyme replacement therapy (ERT), intravenously injected GAA is taken up by lysosomes that have fused with the cell's outer surface, eventually making its way to glycogen-filled lysosomes (bottom).

The Lysosome Connection

Chen has been studying glycogen storage disease, a category of diseases that includes Pompe's, for more than 20 years. (Pompe's disease is sometimes called *glycogen storage disease type 2* or *acid maltase deficiency*; it's one of 10 *metabolic diseases of muscle* covered by MDA's program.)

Early in his career, he recognized that Pompe's disease was going to be a tough nut to crack.

In most glycogen storage diseases, inadequate breakdown of glycogen leads to hypoglycemia, a drop in blood sugar levels that drains the body of energy. In these diseases, supplementing the diet with complex sugars like cornstarch can help maintain blood sugar levels and control symptoms.

But the symptoms of Pompe's disease aren't related to hypoglycemia; instead, they're caused by the accumulation of glycogen itself. GAA is one of many enzymes found in *lysosomes*, compartments inside cells that "clean house" by trapping and degrading glycogen and other chemicals. Without GAA, glycogen builds up inside lysosomes and ruptures them, an effect that's especially damaging to muscle, which naturally makes large amounts of the energy-rich substance. (This makes Pompe's disease a glycogen storage disease *and* a lysosomal storage disease, a category that includes Tay-Sachs and Niemann-Pick diseases.)

Chen has thus focused much of his research on how to deliver GAA to the lysosomes of people with Pompe's disease. This is a tall order, one that might seem to parallel efforts at gene therapy and stem cell therapy for

muscle disease, which haven't yet shown success in the clinic.

But Chen has been able to exploit a key feature of lysosomes: In their business of "housecleaning," they fuse with the outer surface of the cell, allowing them to release their contents and take up substances from the outside. Because lysosomes take in other substances, Chen and others reasoned that intravenously delivered GAA might make its way into the lysosomes of muscle cells.

Yuan-Tsong Chen.
Photos by Cramer Gallimore

Building a Better Enzyme

Enzyme replacement therapy for Pompe's disease wasn't his idea, Chen acknowledges. In clinical trials in the 1970s, patients with the disease were given injections of GAA isolated from human placenta, but the treatment failed.

Later, Chen says, "We learned that in order for the enzyme to work, you need to make a special form of it that can be taken up by the cells in [voluntary] muscles and in the heart. The second critical issue is how to make sufficient quantities of the enzyme for a clinical trial."

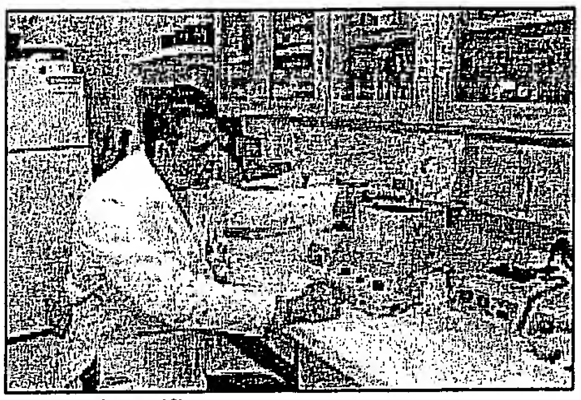
By the 1980s, scientists discovered that for efficient uptake by lysosomes in muscle cells, GAA and other enzymes must have a chemical "tag," called *mannose-6-phosphate (M6P)*. Human placenta had been a plentiful source of GAA, but it produces a version of the enzyme that has very little M6P.

In the 1990s, with advances in molecular biology and funds from MDA, Chen was able to engineer an M6P-laden version of the enzyme — called *recombinant human GAA (rhGAA)* — using a line of cells (*CHO cells*) to produce it in large amounts.

Armed with this new enzyme, Chen formed a collaboration with researchers from Tokyo, who were studying a strain of Japanese quail rendered flightless by naturally occurring Pompe's disease. After three weeks of injections with rhGAA, the birds could fly.

Chen and his team were ready to test rhGAA in babies with Pompe's disease, but first, they needed help from the biotech industry.

"In order to test the therapy in humans," Chen explains, "we needed to make the enzyme in a GMP [good manufacturing


Andy Amalfitano

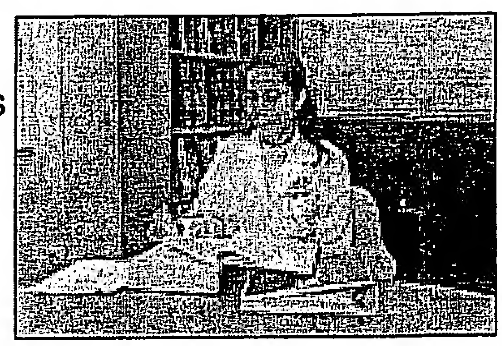
practice] facility, we needed to have every single step documented, and we needed large bioreactors" — incubation chambers for growing the CHO cells that produce rhGAA. "These are things we're not able to do in an academic research lab."

A Lifesaving Treatment

Working first with Synpac, a pharmaceutical company based in Taiwan, and later with Genzyme, a Cambridge, Mass.-based company with a longstanding interest in lysosomal storage diseases, Chen began his first trial of enzyme replacement therapy for Pompe's disease in 1999. The results were published in March 2001 in the journal Genetics in Medicine.

The three babies in the trial, who ranged from 2 months to 4 months old at its inception, had once been expected to die — but all of them are still alive. After about a year of twice-weekly intravenous infusions with rhGAA, all experienced significant reductions in heart size and improvements in cardiac function. Genzyme has continued to supply them with the treatment.

One baby has become "an essentially normal 3-year-old boy," able to walk and to breathe on his own, Chen says. The other two, now 3 and 4 years old, require mechanical ventilation and haven't developed normal motor skills, but they have normal cardiac function, he says.


Priya Kishnani

In 2001, Genzyme and Duke scientists launched a second trial involving eight babies, ranging from 3 months to 14 months old. Five of the infants were studied at Duke and the others were studied at sites in Europe. Details of the results await publication, but Priya Kishnani, the trial's lead investigator at Duke, presented some of her data at a scientific meeting in Dublin in September.

According to her report, all of the babies experienced significant reductions in heart size, two died from complications unrelated to the enzyme, and the remaining six were still alive after about a year of treatment. (For more about one of these toddlers, see "A Time to Celebrate.")

"It's such a fruitful experience to go from a diagnostic approach to a treatment approach for a disease that's considered lethal," Kishnani says. "By no means is this a cure; we don't know the long-term benefits or side effects of the treatment. But there's nothing else out there right now to change the natural course of this devastating disease."

What the Future Holds

This year, Genzyme and the Duke team, led by Kishnani, will begin two larger trials of enzyme replacement therapy for Pompe's disease.

The trials are a final step toward getting the treatment approved by the U.S. Food and Drug Administration, Chen says. One, already under way, is enrolling toddlers between 6 months and 3 years old and the other will enroll babies less than 6 months old. (For more information, contact Genzyme Medical Information at [800] 745-4447.) Each trial will recruit up to 16 patients and will test a different version of rhGAA than that used in the previous trials.

Genzyme, which has made Pompe's disease its largest research and development effort since its founding 21 years ago, now has an arsenal of rhGAA types. The company began testing enzyme replacement therapy for Pompe's disease in 1998, through a joint venture with Pharming, a Dutch biotech company. Scientists from the two companies genetically engineered rabbits to produce rhGAA in their milk, and had begun testing this "transgenic" rhGAA in patients with the infantile and juvenile forms of Pompe's disease. In 2001, Pharming went into receivership and Genzyme acquired the rights to the transgenic rhGAA.

Genzyme acquired another type of rhGAA, made in CHO cells like Chen's, when it bought the Princeton, N.J., company Novazyme Pharmaceuticals.

Recently, Genzyme has developed a fourth version of rhGAA with "improved scalability," meaning it can be produced in larger quantities than previous versions. This is the enzyme that Duke researchers will test in upcoming trials; once a sufficient amount of the enzyme is available, they hope to test it in adults with Pompe's disease.

Looking to the more distant future, scientists at Genzyme and Duke are also investigating gene therapy for Pompe's disease. One potential benefit of this approach is "a decreased need for frequent infusions of the enzyme. You could envision a gene therapy treatment that would only be required yearly," says Andy Amalfitano, a co-investigator in the enzyme replacement therapy trials.

In fact, Amalfitano says, "Pompe's disease may be one of the best diseases to consider treating by gene therapy... because we have an opportunity to treat every muscle in the body without inserting the [GAA] gene into every muscle." A virus could be used to deliver GAA to the liver, which could then release the enzyme into the bloodstream, Amalfi-tano explains. In MDA-funded experiments at Duke, he's used this approach to restore GAA activity to the muscles of mice and quail with Pompe's disease.

Editor's Note: Until now, MDA and Genzyme have made independent efforts to support the development of enzyme replacement therapy for Pompe's disease. In November, MDA and Genzyme staff met at MDA's National Headquarters in Tucson, Ariz., and discussed plans to collaborate on future research.

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EXHIBIT C

ORIGINAL PAPER

Arnold J.J. Reuser · Hannerieke Van den Hout
Agnes G.A. Bijvoet · Marian A. Kroos
Martin P. Verbeet · Ans T. Van der Ploeg

Enzyme therapy for Pompe disease: from science to industrial enterprise

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Abstract Pompe disease or glycogen storage disease type II (OMIM 232300) is a metabolic myopathy with a broad clinical spectrum. Generalised muscle weakness combined with cardiomegaly presents within the first 3 months after birth, if the lysosomal α-glucosidase (AGLU) deficiency is complete. Residual enzyme activity prevents cardiac involvement and delays onset of muscle weakness. Enzyme therapy, by intravenous administration of acid AGLU, aims to supplement the missing enzyme activity. At the SHS symposium on Glycogen Storage Diseases Type I and II, in Fulda, two interim accounts were given of studies on the efficacy of enzyme therapy for Pompe disease; one with recombinant human acid AGLU produced in Chinese hamster ovary cells and the other with the same enzyme produced in the milk of transgenic rabbits. *Conclusion:* this review focuses on the latter study, discusses the scientific, technological and commercial aspects of the enterprise, and addresses the prospects and challenges of enzyme therapy for Pompe disease.

Keywords Acid maltase deficiency · Enzyme therapy · Glycogenosis · Lysosomal storage disease · Transgene technology

Abbreviations *AGLU* α-glucosidase · *CHO* Chinese hamster ovary · *CRIM* cross-reactive immunological material · *M6P* mannose-6-phosphate

A.J.J. Reuser (✉) · A.G.A. Bijvoet · M.A. Kroos
Department of Clinical Genetics,
Erasmus University Rotterdam, PO Box 1738,
3000 DR Rotterdam, The Netherlands
E-mail: reuser@ikg.fgg.eur.nl
Tel.: +31-10-4087153
Fax: +31-10-4089489

H. Van den Hout · A.T. Van der Ploeg
Department of Paediatrics, Division of Metabolic Diseases,
Sophia Children's Hospital, Rotterdam, The Netherlands

M.P. Verbeet
Institute of Chemistry, Leiden University,
Leiden, The Netherlands

Enzyme therapy in historic perspective

Over the past 35 years we have learned what it takes to bring enzyme therapy for lysosomal storage disorders into practice. The concept of enzyme therapy is built on the key function of lysosomes in cell and tissue renewal. Macromolecular compounds, even whole cell organelles like mitochondria, are recycled by the lysosomal system (Fig. 1). Materials derived from the intra-cellular space are sequestered by membranes and delivered through fusion of newly formed autophagic vacuoles with lysosomes. Extra-cellular materials are taken up by bulk or receptor mediated endocytosis and are delivered by fusion of endosomes with lysosomes. Once inside lysosomes, the material is degraded by one or a combination of several lysosomal hydrolases. Some of these hydrolases are assisted by activator proteins [23].

The role of lysosomes in cellular pathology became evident in 1963 with the discovery of acid α-glucosidase (AGLU) deficiency as primary defect in Pompe disease or glycogen storage disease type II [13]. Knowing the function of lysosomes, it was envisaged that patients with lysosomal storage disorders could be treated by administration of the missing enzyme that would find its way to the lysosomes via endocytosis (Fig. 1) [9]. Expectations were high and regulations concerning the performance of clinical studies were less strict in those days than they are at present. The first attempt at enzyme therapy dates from 1964 and involved treatment of a patient with Pompe disease with acid AGLU from the fungus *Aspergillus niger* [5]. Similar experiments followed using enzyme preparations from various other sources [30]. A slight increase of acid AGLU activity in liver was obtained in some of these studies upon intravenous infusion [19], but not in muscle. Reduction of liver lysosomal glycogen only was obtained with high enzyme doses over long periods of time. In all instances, lack of ultimate effect and occurrence of serious side-effects terminated the treatment.

In the period 1965–1980, numerous reports were published about enzyme replacement therapy in several of the lysosomal storage diseases, but most results were basically negative [30]. Some important facts became evident. Enzyme preparations from non-human sources are antigenic. The blood brain barrier cannot be crossed, so that patients with central nervous system involvement cannot be treated for mental and motor dysfunction.

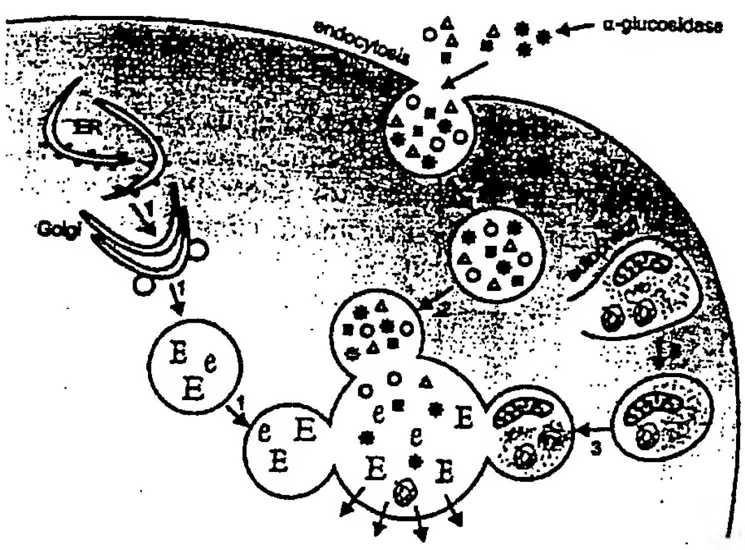

Fig. 1. Enzyme replacement therapy in lysosomal storage diseases. Newly synthesised lysosomal enzymes enter the lumen of the endoplasmic reticulum co-translationally. They are glycosylated, folded, and equipped with the M6P recognition marker as lysosomal targeting signal (route 1). Inside lysosomes, they catalyse the digestion of biological compounds that are delivered by endocytosis (extracellular) (route 2) and autophagy (intracellular) (route 3). Inherited deficiency of lysosomal enzymes leads to lysosomal storage diseases. Lysosomal enzymes administered to cells are taken up via bulk (inefficient) and receptor mediated (efficient) endocytosis and are delivered to the lysosomes where they can supplement the missing enzyme. (*E* enzymes, *ER* endoplasmic reticulum)

Advanced technology is required to secure substantial enzyme supplies over long periods. Around 1980, the focus shifted from enzyme therapy to bone marrow transplantation [15].

A few people pursued the original concept and gave enzyme therapy a second and better chance by applying newly acquired knowledge on receptor mediated endocytosis. Ashwell and Morell [2] were among the pioneers to demonstrate the role of the asialoglycoprotein receptor (galactose) in uptake of glycoproteins by hepatocytes. The mannose receptor was shown to facilitate uptake of proteins with mannose-terminating carbohydrate side chains by Kupffer cells and macrophages of spleen and bone marrow [29], and fibroblasts were found to exchange lysosomal proteins via the mannose 6-phosphate (M6P) receptor [14, 21]. Brady and his group [3] were the first to demonstrate the potential of receptor mediated enzyme therapy for lysosomal storage disease in a patient with Gaucher disease. The glucocerebrosidase used in the study was purified from human placental tissue. The complex carbohydrate side chains were trimmed by sequential action of neuraminidase, galactosidase and N-acetyl-glucosaminidase in order to expose mannose residues and thereby target the enzyme to Kupffer cells and macrophages in spleen and bone marrow; the major sites of lysosomal glycolipid storage in Gaucher disease. The study turned out successful enough to convince scientists, patients and industry of the feasibility of receptor mediated enzyme therapy for Gaucher disease [4].

Enzyme therapy for Pompe disease

We have worked along parallel lines to investigate the feasibility of receptor mediated enzyme therapy for Pompe disease. Table 1 lists the critical events by date.

Table 1. Enzyme therapy for Pompe disease from science to industrial enterprise

Event	Production	
	Milk enzyme	CHO enzyme
Enzyme therapy: proof of principle	[35]	
AGLU cDNA cloning	[16]	
AGLU gene cloning	[24] [17]	
Production	[25] [6]	[11]
Testing in animals	[7] [7] [8]	[36] [36] [22] [7]
Industry enters the scene	Pharming (1991) Genzyme (1998)	Synpac (1994) KDL BioTech (2000) Genzyme (2000) Pharming (2000) Novazyme (2000)
Orphan drug designation	1996	1997
Phase I trial	1998	-
Phase II trial	1999	-
Phase I/II trial	-	1999
Phase II trial data	[31]	[1]
Phase II/III trial	Not planned	May 2001

The target tissues in Pompe disease are muscle and heart. Patients have the same acid AGLU deficiency in all tissues, but the lysosomal glycogen accumulation and the symptomatology are largely restricted to skeletal muscle when the residual enzyme activity is 5%–25% of the normal range. At lower activity levels, heart and other tissues become increasingly involved. There is typically a clinical spectrum from early onset very severe to late onset mild disease. Affected infants have cardiomegaly around birth. They present as floppy babies and die usually before 1 year of age due to cardiorespiratory failure. Onset of symptoms is delayed, and cardiomegaly prevented by low levels of residual AGLU activity. In extreme cases, the skeletal muscle weakness may remain obscure until the sixth decade [27].

Our initial studies on the feasibility of enzyme replacement therapy for Pompe disease were directed towards establishing the presence of M6P receptors on cardiomyocytes and skeletal muscle cells and testing whether these could be employed to facilitate uptake of acid AGLU [26]. To this end, muscle biopsies of patients were dissociated with collagenase and trypsin, and myoblasts (satellite cells) were taken into culture. It turned out no problem to correct the lysosomal glycogen storage in these cells by addition of M6P-containing acid AGLU to the culture medium [32, 33, 34]. Herewith, the first requirements were fulfilled, but the experimental set-up does not mimic the reality in detail. When enzyme therapy is performed via intravenous infusion, the muscle cells do not come into direct contact with the enzyme like they do in tissue culture. The endothelial barrier of the capillaries needs to be crossed and the interstitial connective tissue (endomysium) needs to be passed. In this respect, the situation in Pompe disease is far different from that in Gaucher disease where there are no barriers between the Kupffer cells and the enzyme in the circulating blood. In Fabry disease the situation also is favourable compared to Pompe disease because the endothelial cells are a primary target. Nevertheless, we did obtain uptake of acid AGLU in muscle and heart of mice after intravenous administration of M6P-containing AGLU from bovine testis. The uptake of AGLU without M6P was less [35].

Technical challenges and financing

It took another 8 years before the first clinical trial of enzyme therapy in Pompe disease started. The time was spent on the development of technology to produce therapeutic grade recombinant human AGLU on a large scale. Therapy for Gaucher disease started with the heroic action of Genzyme Corporation (Boston, USA) to produce tailor-made glucocerebrosidase from tons of human placentas. The investment paid off for all parties. Patients with non-neurological forms of Gaucher disease (type 1) obtained an effective medicine [12]. Scientists were pleased because their ideas were realised. The company had shown its strength by bringing a new drug on the market and obtained returns.

The Orphan Drug Legislation, lending certain attractive rights to companies marketing medicines for rare diseases, has played an important role in this development. The past 5 years have shown concerted action of scientists, patients, industry and investors to be a golden formula for developing enzyme therapy for lysosomal storage diseases. Belief in enzyme therapy has returned. More importantly, the first reports confirming efficacy have appeared and clinical trials are ongoing for at least four of the lysosomal storage diseases [1, 10, 20, 28, 31]. In all instances, the protocols are directed to receptor-mediated tissue targeting, and recombinant DNA technology is applied for controlled large-scale enzyme production. The investment climate is excellent. The market seems profitable enough to have several companies competing for the same product. Both TKT (Boston, USA) and Genzyme Corporation have launched enzyme therapy for Fabry disease. Four companies are presently engaged in the development of enzyme therapy for Pompe disease. Genzyme/Pharming/Synpac as conglomerate and Novazyme (Oklahoma City, USA) as newcomer on the lysosomal disease market. As of August 2001, Genzyme and Novazyme have merged.

Back in 1991 there was no company strong and experienced enough to take up the challenge of producing recombinant acid AGLU for the treatment of Pompe disease, but research continued. Two Chinese hamster ovary (CHO) cell lines expressing high levels of recombinant enzyme were produced in university centres using the same AGLU cDNA but different vector systems [11, 36]. Using our CHO cell line, we produced a sufficient amount of enzyme to deliver proof of the principle of enzyme therapy in a mouse model of Pompe disease [7]. Simultaneously, therapeutic effect was shown in Japanese quails with the disease using the other CHO cell line [22]. Meanwhile, a completely different technology emerged which involved the production of medicines in the milk of transgenic animals [18]. Genomic DNA constructs are typically used in this production process in contrast to cDNA constructs employed in CHO cells (Fig. 2). The acid *AGLU* gene is linked to the promoter region of the bovine α_{S1}-casein gene that promotes high level expression in epithelial cells of the mammary gland. The construct is introduced in the animals' genome by injection into the pronucleus of fertilised oocytes. Embryos are implanted in foster mothers and a line of transgenic animals is obtained by germline transmission. The enzyme is harvested from the milk. Both production systems require downstream processing. The recombinant human acid AGLU from either milk or CHO cell media needs to undergo several rounds of purification before it can be administered intravenously to patients. The end products from CHO cells and milk are very similar in molecular mass (110 kD), and kinetic properties [7, 11, 36]. The carbohydrate composition may vary slightly depending on the enzyme source.

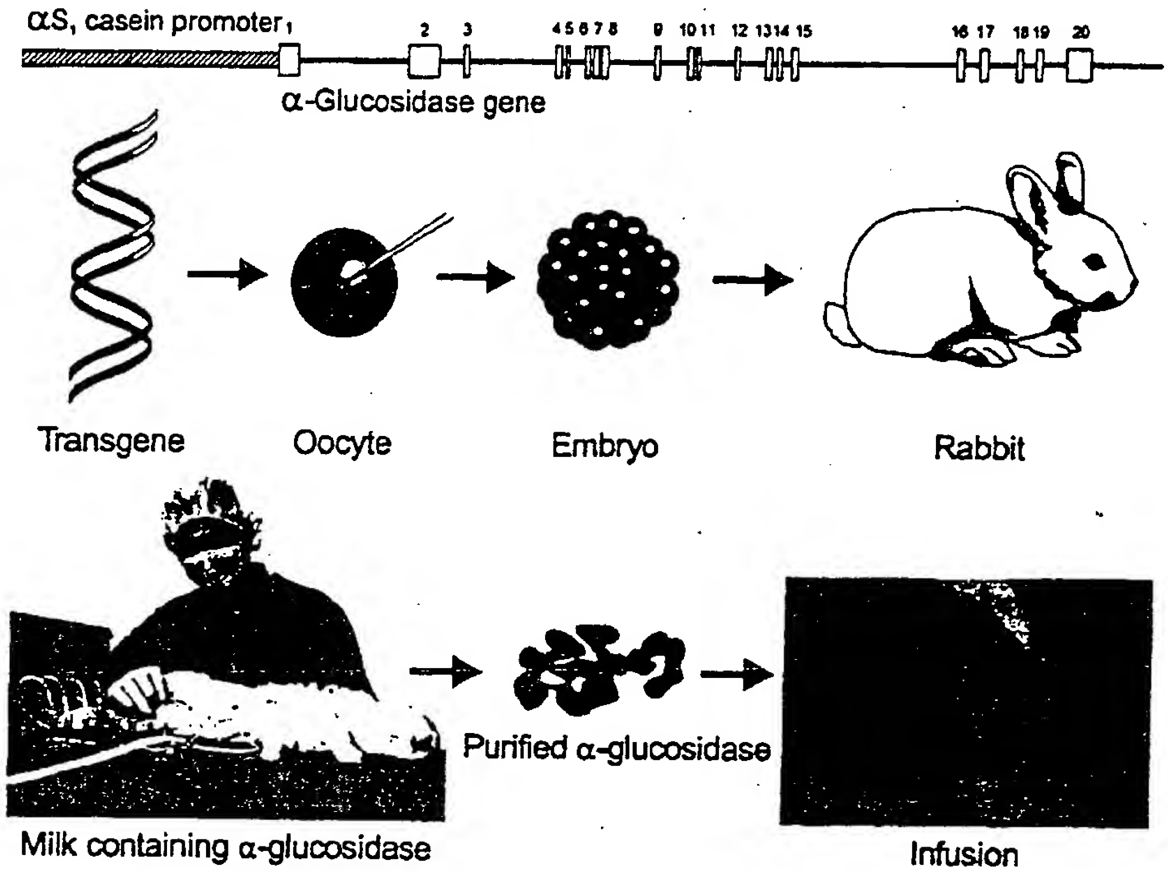

Fig. 2. Recombinant human AGLU from rabbit milk. The human acid *AGLU* gene is fused to 6.3 kb of the bovine α_{S1}-casein promoter and as such injected in the pronucleus of fertilised rabbit oocytes to generate transgenic founders. A transgenic line is obtained by breeding. Females produce recombinant human enzyme in the mammary gland during lactation and secrete the product in their milk. The enzyme is extracted from the milk in several purification steps and administered intravenously to the patients

Recombinant acid AGLU from CHO cells and mouse milk, both produced in our own laboratory, are equally efficiently taken up by cultured fibroblasts of patients with Pompe disease via the M6P receptor. However, the same enzyme from rabbit milk is taken up less efficiently by cultured fibroblasts. Interestingly, the uptake of the two enzymes by the target tissues of mice is not consistently different (unpublished results). More extensive studies are needed to verify these initial findings.

Clinical studies

Recombinant human acid AGLU from both sources were finally tested in the clinic (Table 1). Dr Chen of Duke University, North Carolina, USA reported at the SHS symposium in Fulda the results of his study with CHO enzyme. We have summarised below the design and outcome of our study in which the enzyme from rabbit milk was used [31].

Study design and outcome

The aim of the study was to test safety and efficacy of recombinant human AGLU from rabbit milk in patients with the severe infantile form of Pompe disease. For inclusion, patients had to have the combination of generalised muscle weakness, cardiomegaly, acid AGLU deficiency and glycogen storage in skeletal muscle. Patients older than 10 months and those who were dependent on artificial ventilation were excluded. Four patients were included; two with an advanced stage of disease (7 and 8 months old), and two younger patients who were in significantly better condition at inclusion (2.5 and 3 months old). The two older patients were practically immobile at the time of inclusion. They required supplemental oxygen and had signs of cardiac instability. One of them became respirator dependent directly after inclusion before the start of treatment. The other patient became ventilator dependent after 10 weeks of treatment during a bout of pneumonia. One of the younger patients had signs of cardiac decompensation and respiratory distress at birth and was fed by nasogastric tube. The fourth patient was diagnosed at birth when he showed cardiomegaly on a chest X-ray film that was taken for disease unrelated indication. Both younger patients manifested axial hypotonia, head lag and slipping through.

The recombinant human AGLU from rabbit milk was administered intravenously in a weekly dose of 15–20 mg/kg (at start of treatment) to 40 mg/kg (at present) and is generally well tolerated. Transient reactions are seen sometimes during infusion, such as fever, malaise, erythematous rash, sweating, flushing and tachycardia. All are manageable without medication. After 12 weeks of treatment with the low dose, the acid AGLU activity in muscle had increased from 1%–2% of normal (before treatment) to 12%–28%, the levels typically measured in late-onset Pompe disease. During the 12 following weeks with high dose, the activity increased to normal levels. After 36 weeks of treatment, we observed improvement in muscle morphology in the younger patient who was in the best condition at the start of treatment. Muscle tissue sections stained less intense for glycogen (PAS staining) and muscle fibres appeared less damaged. Similarly clear

changes were at that time not yet evident in muscle biopsies of the other three patients.

Cardiac changes were monitored by ultrasound. The left ventricular mass index of all patients, exceeding approximately three times normal at inclusion, decreased after start of treatment (up to 25% of baseline for one of the patients). Signs of cardiac instability disappeared in all cases.

All patients gained strength over the first 36 weeks of treatment. They learned to play with toys. The two younger patients perform better than the two older ones. One of the younger patients learned to lift her legs from the surface and touch her feet whilst playing. At present she can sit independently. The other, with the best condition at start of treatment, has learned to sit and crawl at 9.5 months of age. At 12 months he could creep and stand with support of one arm and he could walk at 16 months. Importantly, all patients have well passed the age of 1 year which is more than the average life expectancy of patients with infantile Pompe disease. All four patients receive continuous treatment to evaluate the long-term effect of enzyme replacement therapy on motor and mental development and overall quality of life.

Results of two trials

Comparing the studies with recombinant human AGLU from rabbit milk and CHO cells, there are differences and similarities in outcome. One patient in each study responds very well. The patient treated with rabbit milk enzyme had a characteristic cardiomegaly at birth. The patient treated with CHO enzyme had a normal baseline cardiac evaluation with a left ventricular mass at two standard deviations above the norm (close to the P98) when treatment was started at 3 months after birth. Obviously, we are confronted with clinical diversity preventing in part the comparison of data. Further, dose level and infusion frequencies were different in the two trials. A second patient in the study with rabbit milk enzyme responds well in that she has acquired the ability to roll over and sit and has remained ventilator independent over the first 72 weeks. The patient is homozygote for the deltaT525 mutation and does not produce endogenous acid AGLU. Her progress demonstrates that a cross-reactive immunological material (CRIM) negative status is not a priori inhibitory for successful treatment. In contrast, the two CRIM negative patients in the trial with CHO enzyme were said to respond initially well to the treatment, but their condition declined when high antibody titres developed against the recombinant enzyme. The other patients in both trials are CRIM positive to some extent. The difference in antibody response is multi-interpretable. First of all, the enzyme preparations used are probably not identical with respect to precise molecular structure, notably the carbohydrate composition. Second, the two preparations may contain various types and degrees of impurities and are formulated differently. This may affect their immunogenicity. Moreover, the dosing regimen was different at the two test sites. All these factors, separate or together, may explain why CRIM negative patients respond differently to the two enzyme preparations.

Prospects and challenges

The positive effects of enzyme therapy for Pompe disease are too strong to deny. For the benefit of patients, enzyme therapy ought to be brought to the market. It requires a Phase III trial to deliver final proof of therapeutic effect. The current pilot studies with rabbit milk and CHO enzyme indicate that quick and convincing results can be obtained by extension of studies in patients with the infantile form of Pompe disease; but, at the same time, it seems inevitable that not all included patients will respond equally well. The problem can in part be managed by careful definition of inclusion and exclusion criteria and proper dosing. A second challenge is to prove efficacy of enzyme therapy in late onset Pompe disease. The milder affected patients live longer and are continuously confronted with loss of quality of life. It is essential for them to implement therapy at the earliest possible moment in order to prevent irreversible damage of muscle function. In theory, it is easier to correct the enzyme deficiency in late onset than in early onset disease because the residual AGLU activity is significantly higher in the former than in the latter condition. On the other hand, it has to be awaited whether adult muscle tissue is equally accessible for the enzyme and equally reparable as growing muscle of infants.

As it stands, the prospects of enzyme therapy for Pompe disease are good, but hurdles still need to be overcome. In April 2000, Genzyme-Pharming LLC announced the discontinued development of enzyme replacement therapy with recombinant human AGLU from rabbit milk. The companies stated that they believed production in CHO cells to be quicker. A new study with enzyme from CHO cells was started in May 2001. All together, that is more than 35 years after the first trials were undertaken. Patients, investigators and companies are eagerly awaiting the outcome.

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	Title:	Treatment of Glycogen Storage Disease Type II	
	Docket No.:	2984.1000-004	

Sir:

Transmitted herewith are three (3) originally signed copies of a Brief on Appeal for filing in the subject application. The Brief on Appeal is filed pursuant to the Notice of Appeal received by the U.S. Patent and Trademark Office on October 6, 2003.

1. [X] Appellant hereby petitions to extend the time for filing a Brief on Appeal for one month from December 6, 2003 to January 6, 2004.

2. [ ] A [ ] month extension of time to extend the time for filing a Brief on Appeal from [ ] to [ ] was filed on [ ] with payment of a $[ ] fee.

   [ ] Appellant hereby petitions for an additional [ ] month extension of time for filing a Brief on Appeal from [ ] to [ ].

3. [ ] A Request for Oral Hearing before the Board of Patent Appeals and Interferences is being filed concurrently herewith.

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4. Fees are submitted for the following:

[X]	Extension of Time for one month			$ 110
[ ]	Additional Extension of Time:			
	Fee for Extension	([ ] mo.)	$	
	Less fee paid	([ ] mo.)	- $	
	Balance of fee due			$ 0
[X]	Brief on Appeal			$ 330
[ ]	Other			$
			TOTAL	$ 440

5. The method of payment for the total fees is as follows:

[X] A check in the amount of $440 is enclosed.

[ ] Please charge Deposit Account No. 08-0380 in the amount of $[ ].

Please charge any deficiency or credit any overpayment in the fees that may be due in this matter to Deposit Account No. 08-0380. A copy of this letter is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

David E. Brook, R.N. 22592

By for Elizabeth W. Mata

Elizabeth W. Mata
Registration No.: 38,236
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
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